Modification of Irradiation Effects in the Pigeon, *Columba livia*. II. Effects of Delayed Bone Marrow Implantation.* (26643)

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There is general agreement that grafts of autologous, isologous, homologus and, in most instances, heterologous bone marrow prolong the life of lethally irradiated animals, by preventing death due to damage of the hematopoietic system(1). Marrow transplantation has been of limited therapeutic value, because the majority of irradiated individuals grafted with homologous or heterologous marrow ultimately die from a wasting disease ("homologous or heterologous disease")(2).

Shaw *et al.*(3,4,5), using the heterotransplant system of pigeons and doves, demonstrated that both host-species-specific hemagglutinins and precipitins were detectable in the circulation of pigeon-dove chimeras implanted *immediately* after irradiation. An immunologic response of the implanted marrow directed against the host tissue was suggested in line with the general theories proposed by Ford *et al.*(6).

The lack of success from homologous marrow implantation in irradiated humans(7) may be related to similar mechanisms or to an immunologic response of the regenerated host tissues directed against the antigenically foreign marrow implant(8).

Mathé *et al.*(9,10) reported remarkable success, however, in 4 of 5 Yugoslavians accidentally exposed to large doses of irradiation, with homologous marrow injected 30 days after the irradiation. In each of these cases partial repopulation by the donor erythrocytes occurred followed by chimera reversal (*i.e.*, the host hematopoietic system recovered and replaced the implanted marrow system).

The present report includes findings of experiments in which "immediate" and "delayed" (5 to 7 days post-irradiation) implantation of homologous or heterologous bone marrow was employed in pigeons subjected to lethal total body irradiation.

Material and methods. Dose response curves were established and showed that the effective LD 100/30 for the pigeon (*Columba livia*) was 2,500 rads. The source of the birds, methods of irradiation, marrow preparation and injection were as previously described(3).

A total of 241 pigeons were used: 211 received 2,500 rads total-body and 30 were maintained as unirradiated controls. The birds were separated into the following groups:

Group 1: *Immediate homotransplants*: 40 irradiated birds given single intravenous injection of 2 ml of a saline suspension of fresh homologous marrow within 4 to 6 hours after irradiation.

Group 2: *Immediate heterotransplants*: 40 irradiated birds injected intravenously with 2 ml of a saline suspension of fresh heterologous marrow from *Streptopelia risoria* within 4 to 6 hours after irradiation.

Group 3: *Delayed homotransplants*: 50 irradiated pigeons, 30 treated June 1960 (Series A) with 250×10^6 nucleated homologous marrow cells in groups of 10 on days 5, 6 and 7 postirradiation respectively; 20 treated Aug. 1960 (Series B) with the same type and quantity of marrow cells but all on the 5th day after irradiation.

Group 4: *Delayed heterotransplants*: 50 irradiated pigeons treated in the same way as Group 3 except that heterologous dove marrow was injected.

Group 5: *Irradiated controls*: 31 birds injected with 2 ml of sterile saline intrave-

* From the Dept. of Radiology, Medical School, and the Dept. of Genetics (paper No. 837) Agr. Exp. Station, Univ. of Wisconsin. This project was supported in part by the Research Committee of the Graduate School from funds supplied by Wisconsin Alumni Research Foundation and in part by grants to M. R. Irwin and to Halvor Vermund from Dept. of HEW, Nat. Inst. Health, P.H.S.

nously; 10 at the same time as birds in Groups 1 and 2, randomized and housed in the same cages, 11 with Groups 3 and 4, Series A, and 10 with Groups 3 and 4, Series B.

Group 6: *Unirradiated birds*: 30 pigeons injected as in Group 5, 10 with Groups 1 and 2 and 10 each for Series A and B.

The homologous bone marrow was obtained from pigeons whose erythrocytes were distinguishable from those of the host by one or more of the erythrocytic antigenic markers B^g, C^g, and E^g derived from *C. guinea* which were previously designated as B, C and E(11). In certain donor-host combinations the erythrocytes differed in their reaction with the seed extracts of Sieva (*Phaseolus limensis*)(3) and *Bandeiraea simplicifolia*. Each of these 2 phytoagglutinins distinguished between the erythrocytes of individual pigeons; *i.e.*, those whose red cells were agglutinated were designated as Si^+ and Ba^+, respectively, while those with non-reactive red cells were Si^- and Ba^-. Since these erythrocytic properties appeared to be genetically determined and were autonomous (La Bar and Shaw, unpublished), they were used as blood group markers in the same manner as the above mentioned erythrocytic antigenic markers.

Blood samples were drawn for both sera and erythrocytes from the wing veins of all birds on days 0, 4, 6, 8, 10, 12, 20, 28, 30, 35, 40 and 45, or until the birds died. The samples of erythrocytes from Groups 1, 3-A and 3-B were tested with antisera specific for the B^g, C^g and E^g antigenic factors, to reveal the presence or absence of the donor's erythrocytes. They were also tested with saline extracts of the seeds of the Sieva and *Bandeiraea simplicifolia* as well as with antisera specific for the B^1 antigenic factor of *C. livia*, previously called B′(11), to detect the host's or donor's red cells.

Samples of cells from Groups 2, 4-A and 4-B were tested with rabbit anti-*livia* serum (absorbed with cells of *risoria*, rendering it specific for *livia* erythrocytes in contrast to those of *risoria*) to detect pigeon (host) red cells. These samples were also tested for the presence or absence of red cells derived from the bone marrow of the donor with an antiserum (rabbit anti-*risoria* serum absorbed with cells of *livia*) which was reactive with *risoria* erythrocytes, but not with those of *livia*.

The serum samples from birds in Groups 1-6 were tested for possible hemagglutinins against a panel of erythrocyte suspensions from *livia*, *risoria*, and backcross hybrids carrying the B^g, C^g and E^g factors from *Columba guinea*. In addition, each sample of serum from each bird was tested against its own erythrocytes (obtained shortly before the implantation), and against cells of the bone marrow donor to determine if there were hemagglutinins present which were reactive with the erythrocytes of either or both the host or donor prior to implantation. The sera obtained from the heterotransplant groups do exhibit hemagglutinin activity at various times (Table III).

Since antibiotic therapy has been successful in eliminating early deaths, presumably due to bacterial invasion *via* the radiation damaged intestinal tract in both rats(12) and rabbits(13) given lethal total body irradiation, it was employed in these pigeons to reduce early deaths (within 3-5 days). The birds in this study were given Myzon Poultry Builder (terramycin plus vitamins and minerals) in their drinking water starting 10 days prior to irradiation and were maintained on this antibiotic throughout the experimental period. Concentration of the active component was .05 g/liter of Oxytetracycline hydrochloride (Terramycin R).

Results. The data in Table I clearly indicate that survival was increased for both homologous and heterologous bone marrow implants when the implant was delayed 5 to 7 days after irradiation. For the *immediate transplant* groups average survival time was 24.2 days after homologous and only 8.8 days after heterologous bone marrow implantation. In contrast, for the *delayed transplant* average survival time was 46.6 days after homologous and 26.9 days after heterologous implants. *Delayed* bone marrow transplantation in contrast to *immediate* transplantation, resulted in a highly significant increased average survival time from

TABLE I. Survival of Lethally Irradiated Pigeons Receiving Immediate or Delayed Homo- or Heterotransplants.

Treatment Type	Day	Total No. treated	No. of survivors Days postirradiation 4	6	10	14	18	30	45	60	90	Avg survival time, days
HBM (Group 1)	0	40	38	38	23	20	15	9	4	3	2	24.2 ± 3.6
DBM (Group 2)	0	40	34	26	10	6	3	0	—	—	—	8.8 ± .6
HBM (Group 3)	5	10	—	10	9	8	6	4	4	3	2	49.8
Series A	6	10	—	10	8	7	6	3	2	2	2	44.1
	7	10	—	10*	9	7	7	2	2	1	1	42.3
Series B	5	20	—	20	18	15	13	7	6	4	4	47.8
Delay total	—	50	—	50	44	37	32	16	14	10	9	46.6 ± 2.2
DBM (Group 4)	5	10	—	10	9	4	2	2	2	2	1	37.7
Series A	6	10	—	10	8	4	1	0	—	—	—	19.4
	7	10	—	10*	8	6	3	1	1	1	1	27.2
Series B	5	20	—	20	17	10	4	2	2	1	1	25.7
Delay total	—	50	—	50	42	24	10	5	5	4	3	26.9 ± 5.0
Irrad. controls	—	31	31	29	17	11	4	0	—	—	—	13.3 ± 1.0
Unirrad. controls	—	30	30	30	30	30	30	30	30	30	30	

* Day 7.

HBM = Homologous bone marrow, from another pigeon. DBM = Dove (heterologous) bone marrow.

Day 0 = time of transplant after irradiation (approx. 4 to 6 hr). Day 5, 6, or 7 = No. of days between irradiation and time of transplant.

24.2 ± 3.6 to 46.6 ± 2.2 days ($p = .0002$) for homotransplants, and from 8.8 ± 0.6 to 26.9 ± 5.0 days ($p = .0003$) for heterologous marrow implants. There was no significant difference in average survival between the 3 delayed implant groups, although the homologous transplant data suggested that day 5 might be therapeutically somewhat superior to day 7. To answer this question adequately, a larger number of birds would be necessary and an extended time range used.

Of particular interest was the fact that 3 of the pigeons with the *delayed heterotransplants* (Group 4-A) showed prolonged survival time: to the 66th, 126th and 186th day respectively, the 2 latter being shown in Table III (No. 3106.34 and No. 3106.38). This in contrast to a maximum of 19 days survival time for the *immediate heterotransplants* (Group 2), indicated a substantial increase in survival time when the heterotransplant was delayed.

Table II shows the immunologic data on erythrocytic repopulation, which demonstrated that in the *delayed homotransplants* (Groups 3-A and 3-B) which survived to the 60th day, there was only partial donor repopulation and that chimera reversal occurred (see bird No. 3106.6) or at least a proportion of donor cells in circulation reached a maximum and then declined (see bird No. 3106.63). This was not the result previously reported(3), and differed also from the data for birds in the *immediate transplant* Group 1 of this present report. In these latter 2 cases partial and eventual total repopulation of the erythrocytes resulted (see birds Nos. 3100.33 and 3100.44).

Table III contains the immunologic findings for the *delayed* and *immediate heterotransplants* (Groups 2, 4-A and 4-B). It is interesting to note that, whether the dove marrow was implanted immediately or on days 5, 6 or 7, "*in vivo* agglutination" occurred between the 4th and 6th day after such a heterotransplant was made, regardless of the time elapsed after irradiation. As indicated in Table III, when dove marrow was transplanted immediately (Group 2), host-species-specific hemagglutinins appeared in the circulation of the irradiated host and were sufficiently potent to produce a high degree of "*in vivo* agglutination" (titer with

TABLE II. Representative Immunologic Data of Results Obtained in Erythrocyte Repopulation Studies of Irradiated Pigeons Injected with Homologous Bone Marrow on Day 0, 5, 6 and 7.

Bird No.	Blood type of donor	Reagents used to test RBC	Agglutination reactions of erythrocytes* Days postirradiation 0	8	14	20	28	45	60	70	90	Survival time (days)
3100.24	B^g	Sieva	4+	4+								9
Day-0	Si^-	Anti-B^g	0	0								
		Anti-C^g	0	0								
3100.13	B^g & C^g	Sieva	4+	4+	2+	1+	0					30
Day-0	Si^-	Anti-B^g	0	0	2+	3+	3+					
		Anti-C^g	0	0	3+	3+	4+					
3100.33	B^g & C^g	Sieva	4+	3+	2+	1+						21
Day-0	Si^-	Anti-B^g	0	0	2+	3+						
		Anti-C^g	0	±	3+	3+						
3100.44	B^g & C^g	Sieva	3+	3+	2+	1+	0	0	0	0		78
Day-0	Si^-	Anti-B^g	0	0	2+	2+	3+	4+	4+	4+		
		Anti-C^g	0	0	2+	3+	3+	4+	4+	4+		
3106.5	B^g & C^g	Ba-Ext.	0	0	0	1+	2+	±	0	0	0	131
Day-6	Ba^-	Anti-B^g	0	0	0	2+	3+	2+	1+	0	0	
		Anti-C^g	0	0	±	2+	3+	2+	1+	0	0	
3106.63	B^gB^g	Ba-Ext.	0	0	0	0	0	0	0	0	0	124
Day-5	Ba^-	Anti-B^g	0	0	±	2+	3+	2+	2+	1+	± 1+	
		Anti-B^1	4+	4+	3+	3+	3+	3+	3+	3+	3+	
3106.62	B^gB^g	Ba-Ext.	2+	2+	2+	1+						26
Day-5		Anti-B^g	0	0	2+	3+						
		Anti-B^1	4+	4+	3+	1+						
3106.69	B^g	Ba-Ext.	3+	3+								12
Day-5	Ba^-	Anti-B^g	0	0								
		Anti-C^g	0	0								

* Degrees of agglutination were as follows: Complete (4+); approx. 75% (3+); approx. 50% (2+); approx. 25% (1+); detectable but less than 20% (±); and not detectable (0).

pigeon cells ranging from 8 to 512) and persisted until death. The few birds which survived beyond the 10th day showed complete erythrocytic repopulation by the marrow implant (see bird No. 3100.37). This is in complete accord with previous findings(3,4). Total erythrocytic repopulation was not evident when delayed marrow implants were made (Group 4-A and B). Although the host-species-specific hemagglutinins occurred in circulation between the 4th and 6th days after the implantation their titer was somewhat lower (titer with pigeon cells ranging 8 to 64) than those observed in the *immediate heterotransplants* yet sufficiently potent to produce "*in vivo* agglutination," but without all the host's erythrocytes being eliminated. For a period both the host's and the donor's erythrocytes were present in the circulation. Approximately 15 days after implantation, or 20 days after irradiation, the host-species-specific hemagglutinins disappeared. By the 30th day after implantation (35 days after irradiation) donor-species-specific hemagglutinins appeared in the circulation and, although these agglutinins ranged in titer from 8 to 16 with dove cells, no "*in vivo* agglutination" was observed. This was possibly due to the relatively small number of erythrocytes in the circulation derived from the implanted dove marrow. On the 45th day postirradiation chimera reversal was completed, *i.e.*, the only erythrocytes detectable by the agglutination procedure were of the host's (pigeon) specificity (see birds Nos. 3106.34 and 3106.38).

Due to the high radiation tolerance of pigeons (LD 100/30 = 2,500 rads), damage to the intestinal mucosa might be expected to be more severe than in mice. This was illustrated by the relatively large percentage of early deaths (within 3 to 5 days), presumably due to bacterial invasion, obtained in previous experiments(3). Pilot experiments with antibiotic therapy in pigeons have indicated that an increase in survival oc-

TABLE III.* Immunologic Data for 5 of the Irradiated Pigeons Injected with Heterologous Bone Marrow, Showing Each of the Different Types of Results Obtained.

Bird No.	Types of observations	Time blood samples were obtained (days postirradiation) 0	4	6	10	12	20	35	45	Survival time
3100.16	Degree of "*in vivo* agglutination"	0	2+	3+						6
Day-0	Hemagglutinin activity of serum with RBC's of: *livia*	0	2+	3+						
	risoria	0	0	0						
3100.37	Degree of "*in vivo* agglutination"	0	1+	3+	0	0				19
Day-0	Hemagglutinin activity of serum with RBC's of: *livia*	0	1+	3+	4+	4+				
	risoria	0	0	0	0	0				
	RBC activity with: Anti-*livia*	4+	4+	4+	† 0	0				
	Anti-*risoria*	0	1+	3+	4+	4+				
	Isotonic NaCl	0	1+	3+	0	0				
3106.34	Degree of "*in vivo* agglutination"	0	nt	0	1+	2+	1+	0	0	126
Day-6	Hemagglutinin activity of serum with RBC's of: *livia*	0	nt	0	±	3+	3+	0	0	
	risoria	0	nt	0	0	0	0	2+	3+	
	RBC activity with: Anti-*livia*	4+	nt	4+	4+	3+	2+	† 4+	4+	
	Anti-*risoria*	0	nt	0	1+	2+	2+	0	0	
	Isotonic NaCl	0	nt	0	1+	2+	1+	0	0	
3106.38	Degree of "*in vivo* agglutination"	0	nt	0	1+	2+	1+	0	0	186
Day-5	Hemagglutinin activity of serum with RBC's of: *livia*	0	nt	0	2+	3+	2+	0	0	
	risoria	0	nt	0	0	0	0	3+	2+	
	RBC activity with: Anti-*livia*	4+	nt	4+	4+	3+	2+	† 4+	4+	
	Anti-*risoria*	0	nt	0	2+	2+	3+	±	0	
	Isotonic NaCl	0	nt	0	1+	2+	1+	0	0	
3106.68	Degree of "*in vivo* agglutination"	0	nt	0	2+	3+				14
Day-5	Hemagglutinin activity of serum with RBC's of: *livia*	0	nt	0	2+	3+				
	risoria	0	nt	0	0	0				
	RBC activity with: Anti-*livia*	4+	nt	4+	4+	4+	± †			
	Anti-*risoria*	0	nt	0	1+	3+				
	Isotonic NaCl	0	nt	0	1+	3+				

* Symbols for degrees of agglutination same as used in Table II.

† Agglutination in these samples is due to *in vivo* agglutination though it appears that specific antisera increase degree of agglutination.

curred in all irradiated groups (both transplanted and controls) and deaths prior to day 5 were eliminated. Additional studies along this line suggested that even more effective protection against early death was obtained when antibiotic therapy was started 10 days prior to irradiation. The therapeutic effect was not, however, sufficient to give 30 day survival in either the *immediate heterotransplant* or in the irradiated control groups.

Discussion. It appears probable from these data that delaying the implant for a period of 5 to 7 days allowed time for the irradiated host's hematopoietic system to recover (*i.e.*, a portion of the host's hematopoietic cells, though probably in a state of mitotic arrest, was not destroyed by the irradiation and had had time to recover). This would not be the case, however, when immunologically antagonistic donor marrow was implanted immediately. Under these conditions, if the implanted marrow was immunologically competent, it would be expected that the donor's immunologic mechanism would destroy the residual host tissue before recovery was possible. This certainly appeared to be true in the pigeon studies as borne out by the "*in vivo* agglutination"

which occurred in *immediate heterotransplant* pigeon-dove chimeras, apparently resulting from the host-species-specific hemagglutinins present. As previously reported(3, 4), the most plausible interpretation for the existence of these hemagglutinins is that they were elaborated by, or their specificity was directed by, the cells of the heterotransplants. Other reports(14,15,16) also indicate immunologic competence of the donor marrow.

The sequence of changes described suggests that the donor's bone marrow might coexist with the residual hematopoietic tissues of the host, each exerting its own immunologic influence. Furthermore, the evidence suggests that the host tissues might have a selective advantage which tends to result in chimera reversal. Under the conditions which appeared to exist in the *delayed implant* groups, the chances of an acute immunologic reaction sufficient to result in death were reduced. The data in this report, along with information on the Yugoslavians exposed to iradiation by accident and treated with homologous marrow(9,10), indicated that the best therapeutic effects may be achieved if the host's and donor's immunologic responses are modified during a critical period after the implantation period. Such modifications, properly manipulated, could result in chimera reversal so that the final result would be an individual with its own hematopoietic and immunologic systems restored. The donor marrow under these conditions would serve only to supply necessary formed elements and other substances during a period when the host's system is inadequate due to radiation injury. Under certain conditions a balanced chimera would also be expected to survive, *i.e.*, if actively acquired tolerance developed in both the host and the grafted tissue. Chimera reversal or balanced chimerism appears indeed essential for prolonged survival of lethally irradiated animals grafted with "foreign" marrow. Predictability and some degree of control of such reversal by adjusting size of inoculum and time of administration would be a major step toward therapeutic utilization of bone marrow implantation.

Summary. 1. *Delayed* bone marrow *transplantation,* in contrast to *immediate transplantation,* resulted in an increased average survival time of approximately 20 days for both homologous and heterologous marrow in lethally irradiated pigeons. 2. *Delayed homotransplantation* resulted in only partial repopulation by donor derived erythrocytes. Chimera reversal occurred or the proportion of donor erythrocytes in circulation reached a maximum and then declined, while *immediate homotransplantations* resulted in eventual total repopulation by donor erythrocytes. 3. In the heterologously treated pigeons *"in vivo* agglutination" occurred between the 4th and 6th day *after heterotransplantation* regardless of the number of days after irradiation. 4. *Delayed heterotransplantation* did not result in elimination of all the host's ertyhrocytes, as was the case for the *immediate heterotransplants.* However, host-species-specific hemagglutinins were detectable at titers ranging from 8 to 64 with host cells, and sufficiently potent to produce *"in vivo* agglutination". 5. The host-species-specific hemagglutinins, which were present in the *delayed heterotransplants,* disappeared 15 to 20 days after the marrow implant and by the 30th day donor-species-specific hemagglutinins appeared in the circulation. In contrast, the host-species-specific hemagglutinins persisted until death (longest survivor, 19 days) in the *immediate heterotransplants.* 6. Chimera reversal occurred in all 5 of the *delayed heterotransplants* which survived until the 45th day postirradiation. In contrast, the longest survival time after *immediate heterotransplantation* was 19 days and total repopulation by donor erythrocytes occurred in all animals which survived 10 days or longer.

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Received April 24, 1961. P.S.E.B.M., 1961, v107.

Some Pharmacologic Properties of Pangamic Acid (Vitamin B-15).* (26644)

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Pangamic acid (dimethyl-amino-acetyl-gluconic acid) also known as Vit. B-15, was discovered by E. T. Krebs in 1938. The presence of this vitamin has been demonstrated in apricot kernels, rice bran, brewer's yeast, ox blood and horse liver. It is thought to accompany the other members of the Vit. B complex(1,2). Although pangamic acid has been reported to be without pharmacologic properties, it has been suggested for use in treatment of cardiovascular and rheumatic diseases, alcoholism and liver cirrhosis(1. 2,3). This investigation deals with some pharmacologic properties of pangamic acid. Although this compound appears to be structurally dissimilar from thiamine, this investigation demonstrates that pangamic acid shares some of the pharmacologic actions of thiamine(4,5,6).

Methods. Neuromuscular blocking activity was evaluated by using the sciatic nerve-gastrocnemius muscle preparation in the rabbit and chicken. Mature, Dutch rabbits were anesthetized with sodium phenobarbital (200 mg/kg) and bipolar, silver electrodes were placed on the peripheral end of the sectioned sciatic nerve. The nerve was stimulated for 0.2 second with monophasic, square wave pulses of 2 milliseconds duration at a frequency of 250 per second and of supramaximal voltage delivered every 5 seconds. A Grass stimulator (Model S4C) with circuit interrupter was used in all experiments. Semi-isometric recordings were made on smoked kymograph paper. Sixteen animals were used to evaluate the activity of pangamic acid. A linear regression and the dose producing 50% neuromuscular blockade (ED-50) and its 95% confidence interval were calculated as outlined by Finney(7). The neuromuscular effects of pangamic acid were also studied in the chicken using the method described by Pelikan *et al.*(8). The parameters described for the rabbit sciatic nerve-gastrocnemius muscle preparation were also used in this preparation.

The effect of pangamic acid on blood pressure was studied in 20 anesthetized dogs (thiopental soduim 15 mg/kg and barbital sodium 250 mg/kg). Carotid or femoral arterial pressure, electroencephalographic (EEG) and electrocardiographic (ECG) recordings were made. In 5 dogs, the left fore limb was perfused with blood from the abdominal aorta using a constant flow Sigmamotor pump. Injections were made into the perfusing blood or given systemically. Systemic arterial blood pressure and fore limb perfusion pressure were recorded simultaneously. Drug action on the isolated rabbit heart was studied by the clas-

* This investigation supported in part by USPHS Training Grants.