gested that because of the macromolecular size of the heated globulin anaphylaxis was produced, and in this manner the anaphylactic action of endotoxin was enhanced.

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Relation of Blood Group Genotypes to Therapeutic Effectiveness of Bone Marrow Transplants Following X-Irradiation.* (29538)

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The purpose of this study was to determine if the genetic relationship of donor and host blood group genotypes has any effect upon survival of lethally X-irradiated chicks receiving bone marrow transplants and also to investigate the presence and persistence of donor type red blood cells in the circulatory system of the graft recipients during the post-irradiation period.

A number of investigators have established the therapeutic effect of bone marrow transplants following lethal X-irradiation in mice (1-4) and rats(5,6). Similar studies with guinea pigs(1,7) have reported survivors subsequent to lethal X-irradiation and bone marrow transplanting. Autotransfusion of bone marrow in protecting dogs from X-irradiation has been reported by Alpen and Baum(8).

In work with isologous, homologous, and heterologous bone marrow transplantation, Van Bekkum and Vos(6) concluded that the quantity of hematopoietic cells necessary for recovery of lethally irradiated rats and mice depends upon the genetic relationship between donor and host. This was further demonstrated by Urso *et al*(4) in work with rats and mice. Makinodan(9) and Soska *et al*(2) reported that homologous bone marrow injections in lethally irradiated mice merely postponed death. Uphoff and Law(3) confirmed the importance of genetic relationship in work with 2 inbred strains of mice where a known single gene difference existed. The persistence of graft-donor type cells in the blood of lethally irradiated recipients has been studied by a number of investigators. Using agglutinating antisera, Van Bekkum and Vos(6) found a mixed population of donor and host cells in the recipients over an extended interval of time. This has also been demonstrated by other workers using agglutinating techniques(4,10), and by Nowell *et al*(11) using the alkaline-phosphatase test. Gengozian *et al*(12) showed by immunological tests that the thymus glands of lethally irradiated mice protected with rat bone marrow were repopulated by rat type cells, and that this repopulation was complete by 30 days after treatment.

Materials and methods. Chicks of known parentage were produced for this study from a partially inbred line of Single Comb White Leghorns (Texas Line 24). Artificial inseminations were made such that the resulting progeny would express varying degrees of blood group heterozygosity with respect to the 5 blood group systems known to be segregating within the parental stock. This was

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done to allow selection of donor and host individuals of compatible blood group genotypes as well as donor and hosts with incompatibility at one or five blood group loci.

All progeny were blood typed at 16 days of age using isohemagglutinin prepared according to the method described by Fanguy (13). From these tests individuals were assigned an *A, B, C, D,* and *E* blood group genotype according to the nomenclature of Briles(14).

Additional chicks of unknown blood group genotype and parentage were produced, for a second phase of this study, from a non-inbred meat line. This line is known to have no common ancestors with Texas Line 24 within the past 15 years.

Irradiation was delivered by a 220 kv Westinghouse Quadro-Condex Deep Therapy Unit operating at 180 kv, 13 ma, and with a filter system consisting of 0.5 mm Cu plus 1.0 mm Al. The skin to irradiation source was approximately 50 cm and the dose rate was approximately 16.5 r/min as measured in air with a Victoreen dosimeter.

Chicks were contained in a cylindrical cardboard container covered with cheesecloth during the irradiation period. This container was of sufficient size to allow simultaneous irradiation of approximately 20 chicks and still permit a limited amount of individual movement. Irradiated host individuals were given 1800 r of discontinuous X-irradiation which consisted of 300 r increments interrupted by 40-minute intervals without irradiation. Each irradiated lot contained chicks from the control group and from the treatment groups to minimize any error due to variation in irradiation technique.

When approximately 21 days of age, selected hosts were irradiated and at the same time, selected donors were sacrificed. Bone marrow was removed from the femur of sacrificed donors by means of a cannula connected to an aspirator pump. The marrow cells were collected in a trap, suspended in isotonic saline solution, and then homogenized to maximize free cells in the suspension. Fatty tissue was removed following centrifugation, and the desired concentration of cell suspension was obtained by addition of isotonic saline.

Donor cells from birds of the inbred line were identified by individual bird while those cells taken from the meat-line chicks were pooled. All cell samples were transplanted within 4 hours following collection.

Immediately following completion of X-irradiation, intravenous injections of appropriate donor cells were made into the selected host. In Exp. 1 each host received 0.75 ml of a 4% suspension of donor bone marrow cells. In Exp. 2 each host received 0.75 ml of an 8% suspension of donor bone marrow cells. Each control chick received an intravenous injection of 0.75 ml of saline solution.

After treatment all groups were randomly distributed within battery brooders. For donor-host combinations where blood group genotypes differed, the presence and persistence of donor red cells in the vascular circulation of the host were determined by agglutination tests using donor-specific isoantibodies. These determinations were made on survivors at 5, 10, 15, 20, 25, 30, 40, 50, 60, and 80 days post-irradiation.

Four groups of chicks were involved in Exp. 1: (1) 16 irradiated control chicks; (2) 16 irradiated host chicks that received pooled bone marrow cells from the unrelated meat-line donor chicks; (3) 20 irradiated hosts that received bone marrow cells from a single donor of the same line and with an identical blood group genotype; and (4) 20 irradiated hosts that received bone marrow cells from a single donor of the same line but with an identifiable different blood group genotype.

Six groups of chicks were involved in Exp. 2: (1) 48 irradiated control chicks; (2) 19 donor-host combinations which were incompatible at all 5 blood group loci; (3) 21 donor-host combinations which were full-sibs and were incompatible at one blood group locus; (4) 27 donor-host combinations which were non-sibs and were incompatible at one blood group locus; (5) 26 donor-host combinations which were non-sibs but were of identical blood group genotypes; and (6) 37 donor-host combinations which were full-sibs

TABLE I. Effect of Donor-Host Blood Group Genotype Relationship on Survival Following X-irradiation (Exp 1).

Days post-irradiation	Mortality (No.) Irradiated controls (16 chicks)	Donor-host different breed (16 chicks)	Donor-host compatible at all identified loci (20 chicks)	Donor-host incompatible at all identified loci (20 chicks)
0– 1	0	0	0	0
2– 7	4	7	3	7
8–12	10	7	6	12
13–30	1	2	5	1
Survivors	1	0	6	0

and of identical blood group genotypes.

Results. A summary of survival data for Exp. 1 is presented in Table I and Fig. 1. Comparison of the survival of the irradiated controls with the group having donor and host of different breeds and with the group having donor and host of the same inbred line but of incompatible blood type at all 5 blood group loci shows them not to differ significantly. These 3 groups were pooled and their survival rate (1.96%) compared with the survival rate (30.0%) of the group in which donor and host were from the same inbred line and of compatible blood types. This difference was found to be highly significant ($P<.01$).

Table II and Fig. 2 present results of Exp. 2 in which a larger volume of donor cells were used. Again there was essentially no difference in survival rate of the control group (25.0%) and the group in which donor and host were incompatible at all 5 blood group loci (21.05%). The highest survival rate (83.78%) was obtained in the group in which donor and host were full-sibs and also of compatible blood type. This was in contrast to 69.23% survival for the group in which donor and host were non-sibs but of compatible blood type. For the 2 groups in which donor and host were incompatible at only one blood group locus, survival rates were 42.86% and 44.44% for the sibs and non-sib donor-host combinations, respectively. In Table III is given the probability of the 30-day survival rates for the 6 treatment groups differing significantly based on the contingency table chi-square method of Snedecor and Irwin(15).

Since no significant difference existed between survival rates of irradiated hosts treated with bone marrow homogenate from sib or non-sib donors incompatible at only one locus, the 2 groups were pooled and the effects of the individual blood group systems are shown in Table IV. The 30-day survival rates for donor-host incompatibility at the *A*, *C*, and *D* blood group loci were 50.00%, 53.85% and 62.50%, respectively. These are in contrast to 13.33% survival of hosts receiving bone marrow transplants from donors of incompatible *B* system genotypes. Statistical analysis shows this group to be significantly different from the other 3 groups ($P<.01$).

TABLE II. Effect of Donor-Host Blood Group Genotype Relationship on Survival Following X-irradiation (Exp 2).

		Donor-host relationship				
	Irradiated controls	Incompatible at all identified blood loci	Sibs incompatible at one blood locus	Non-sibs incompatible at one blood locus	Non-sibs compatible at all identified blood loci	Sibs compatible at all identified blood loci
No. chicks	48	19	21	27	26	37
No. 30-day survivors	12	4	9	12	18	31
% 30-day survivors	25.0	21.1	42.9	44.4	69.2	83.8

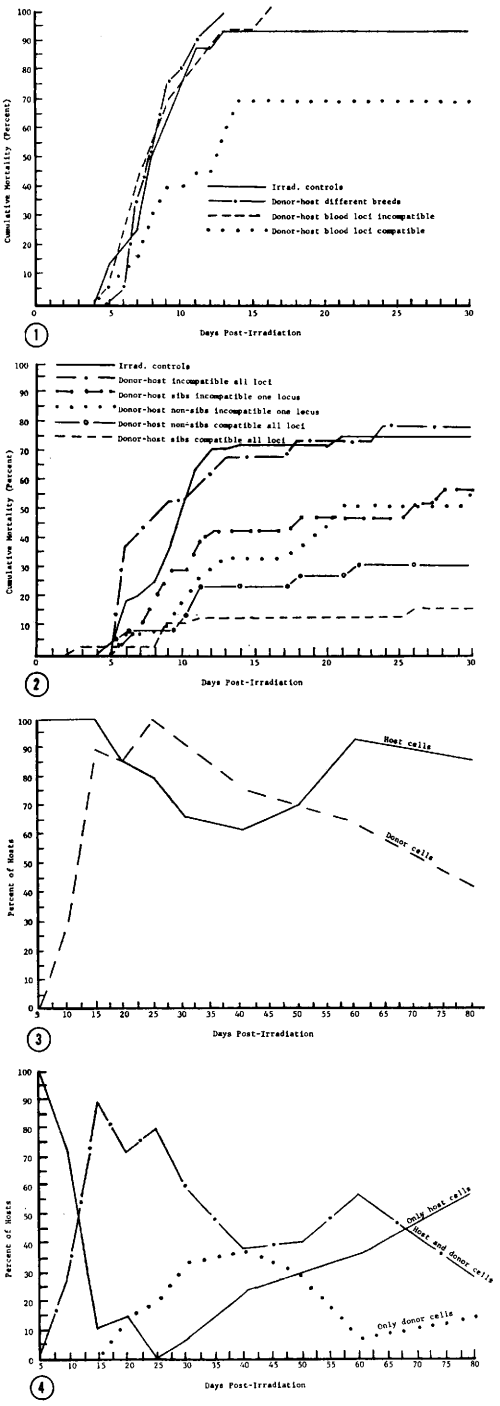

FIG. 1. Effect of donor-host blood group genotype relationship on survival of 21-day-old chicks following bone marrow transplants given immediately after 1800 r discontinuous x-irradiation (Exp. 1).

FIG. 2. Effect of donor-host blood group genotype relationship on survival of 21-day-old chicks following bone marrow transplants given immediately after 1800 r discontinuous x-irradiation (Exp. 2).

FIG. 3. Percent of chicks circulating host and donor red cells subsequent to 1800 r x-irradiation and bone marrow transplantation.

FIG. 4. Percent of chicks expressing blood type of host only, donor only, and combination of host and donor subsequent to 1800 r discontinuous x-irradiation and bone marrow transplantation.

The number and percentage of host birds found to be circulating detectable donor and host type red cells at various time intervals following irradiation and bone marrow transplantation are shown in Table V and Fig. 3, respectively. In no instance were donor red cells detected in the circulatory system of the host by 5 days post-irradiation, but the cells were detectable in the blood of 28.6% of the hosts by 10 days post-irradiation. The percentage of hosts circulating donor type red cells increased rapidly on subsequent tests, reaching 100% by 25 days post-irradiation among the survivors. The decline in number of hosts showing the presence of donor type red cells was gradual for the remainder of the tests, reaching a low of 42.9% by 80 days post-irradiation.

Through the first 15 days of post-irradiation 100% of the birds showed the presence of host type cells. This declined to a low of 61.5% by 40 days post-irradiation and then gradually increased to 85.7% by 80 days post-irradiation. During the first 15 days following irradiation and bone marrow transplantation no host birds were found to be circulating exclusively donor type cells (Fig. 4). However, by 20 days post-irradiation 14.3% of the host birds were found to be circulating only donor type cells. This increased to 38.5% by 40 days post-irradiation, decreased to 7.1% by 60 days post-irradiation and then increased to 14.3% on the final test at 80 days.

At 5 days post-irradiation 100% of the irradiated bone marrow treated hosts were circulating exclusively host type red cells (Fig. 4). This decreased to 71.4% by 10 days post-irradiation and then to 10.5% by 15 days, 14.3% by 20 days, and to 00.0% by 25 days post-irradiation. There was a gradual increase in later tests and at 80 days

TABLE III. Probability of Differences in 30-Day Post-Irradiation Survival Rates Being Due to Treatment.

Treatment groups	Donor-host relationship: Incompatible at all identified blood loci 2	Sibs incompatible at one blood locus 3	Non-sibs incompatible at one blood locus 4	Non-sibs compatible at all identified blood loci 5	Sibs compatible at all identified blood loci 6
1*	.20	.80	.90	.99	.999
2		.80	.90	.999	.999
3			.00	.90	.999
4					.999
5					.80

* Controls.

post-irradiation, 57.1% of the birds tested were circulating exclusively host cells. The donor-host combination of cells first was detected at 10 days post-irradiation (28.6%) and reached a high-level (89.5%) by 15 days post-irradiation. Thereafter, the percentage dropped to 28.6% by 80 days post-irradiation.

TABLE IV. Effect of Specific Blood Group Loci upon Survival.

Incompatible donor-host blood locus	No. host birds tested	No. survivors to 30 days post-irrad	% Survivors
A	4	2	50.00
B	15	2	13.33*
C	13	7	53.85
D	16	10	62.50

* Significantly different from other three loci ($P < .01$).

Discussion. The foregoing experimental results extend to fowl the observations of Van Bekkum and Vos(6) and others(2-4,9) who worked with isologous, homologous and heterologous bone marrow transplants in lethally irradiated rats, mice and guinea pigs. Survival of irradiated hosts receiving bone marrow transplants from donors of a different breed or of completely incompatible blood type was essentially the same as those irradiated individuals receiving no bone marrow transplants. This low survival rate, as compared to the high survival rate of irradiated hosts receiving transplants from donors of identical identified blood group genotypes is indicative of the importance of donor-host blood group genotypes to survival. Within the treatment groups receiving bone marrow transplants from hosts of identical blood type the survival rate was about 15% greater when donor and host were full-sibs as compared to donor-host combinations of non-sibs. This would suggest that genetic factors other than blood groups are also important to survival.

The low therapeutic effect of bone marrow transplants from genetically divergent hosts is in contrast to the high early survival rates reported by Urso *et al*(4) in work with rats and mice. It is possible that this could be a function of cell concentration used in transplantation since survival of certain treatment groups in this study were increased approxi-

TABLE V. Persistence of Host and Donor Red Cells in Host Individuals During Post-Irradiation Period.

	Days post-irradiation: 5	10	15	20	25	30	40	50	60	80
Total No. survivors tested	28	28	19	14	10	15	13	10	14	7
Distribution of survivors										
No. circulating host cells	28	28	19	12	8	10	8	7	13	6
" " donor cells	0	8	17	12	10	14	10	7	9	3
" " only host cells	28	20	2	2	0	1	3	3	5	4
" " only donor cells	0	0	0	2	2	5	5	3	1	1
" " both host & donor cells	0	8	17	10	8	9	5	4	8	2

mately 45% when cell suspensions were increased from 4% to 8%.

Of the 4 blood group systems investigated, compatibility of donor and host at the *B* locus was found to be most critical to host survival. In 100% of the host individuals receiving transplants from donors incompatible at the *A, C,* or *D* blood group loci, donor-type cells were found to be circulating at some time during the post-irradiation period. Where donor and host were incompatible at the *B* locus only one bird was found to be circulating donor-type cells. This success of incompatible *A, C* and *D* system bone marrow grafts and the failure of incompatible *B* system grafts is similar to results of skin homograft studies in birds reported by Gleason and Fanguy(16). They found that donor-host incompatibility at the *D* and *E* blood group loci did not influence homograft survival while incompatibility of donor and host at the *B* locus resulted in 100% homograft rejection. They also found *B* locus incompatibility accelerated homograft rejection. In this present study it was found that hosts receiving incompatible *B* locus transplants survived an average of 23 days post-irradiation as compared to 55 days, 46 days and 69 days survival for donor-host incompatibility at the *A, C* and *D* loci, respectively (hosts alive at 100-day post-irradiation not included).

The general pattern of predomination of host cells during the early period of post-irradiation, predomination of a donor-host complex of cells during the 15- to 65-day period of post-irradiation and a return to predominantly host-type cells during the latter post-irradiation period is in general agreement with data reported by Ford(17) and Urso *et al*(4). However, in neither of the above mentioned investigations was the persistance of donor cells as high as was found in this study.

Gengozian *et al*(12) reported that thymus glands of mice were completely repopulated with rat-type cells within 30 days following irradiation and cell injection. In this investigation, although 93.3% of the hosts were circulating donor-type red cells by 30 days post-irradiation, only 33.3% had exclusively donor cells. It should be pointed out that this value, as are others reported in this study, is subject to error in red cell identification technique. More specifically, it is conceivable that a cell type, either donor or host, could be present in a blood sample in such low concentration that it was not detectable by hemagglutination techniques.

Summary. Chicks of known blood group genotype and parentage produced from a partially inbred line of Single Comb White Leghorns were subjected to 1800 r of discontinuous X-irradiation when approximately 21 days of age. Irradiation was administered at the rate of 16.5 r/minute in increments of 300 r interspersed with 40-minute intervals without irradiation. Immediately following irradiation hosts received intravenous injections of bone marrow homogenate obtained from sacrificed donors having various degrees of blood group genotype relationship with the respective hosts. Thirty-day post-irradiation mortality of hosts receiving transplants from donors of a different breed or of the same breed but of incompatible *A, B, C* and *D* blood group genotype was essentially the same as the irradiated controls receiving no bone marrow transplants. These groups averaged 7.3% survival as opposed to 66.3% survival for hosts receiving transplants from donors of identical blood type. When donor and host were full-sibs in addition to having compatible blood type the survival rate increased to 83.8%. Incompatibility of donor and host at the *A, C* and *D* blood group loci resulted in essentially the same levels of mortality; the average being 42.42%. This is in contrast to 86.67% mortality of hosts having donors of incompatible *B* system genotype. Hemagglutination tests showed the majority of the hosts to be circulating red cells of the host-type by 10 days post-irradiation, a mixture of donor and host types from 15 days through 60 days post-irradiation and host-type by 70 days post-irradiation. These tests also showed 38.5% of the hosts to be circulating only donor-type cells by 40 days post-irradiation and one individual still exhibited only donor-type cells at 80 days. Donor-type

cells were detected in 100% of the hosts having donors of incompatible *A*, *C* or *D* blood type. Where incompatibility at the *B* locus was involved, only one host was found to be circulating donor-type cells during the post-irradiation period.

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Collagen Synthesis and C^{14}-Labeled Proline Uptake and Conversion to Hydroxyproline in Steroid-Treated Granulomas.* (29539)

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Although it has been shown by morphological and histochemical studies that certain steroids depress fibroblastic activity and collagen formation(1-5), there have been few chemical determinations of collagen metabolism following steroid treatment. Robertson and Sanborn obtained a reduction in total collagen concentration in subcutaneous granulomas with large dosages of an 11-oxy steroid(6), while Jorgensen(7) found that steroid treatment decreased the proliferation of dermal granulation tissue, but not its collagen concentration. Studying the specific collagen fractions, Houck showed that sham adrenalectomy and cortisol administration decreased the total neutral soluble and insoluble collagen concentration in rat skin but increased the acid-soluble content(8). The following studies were undertaken to determine the influence of certain steroids on: 1) granuloma collagen content, and 2) proline incorporation into the salt-soluble collagen fraction and its subsequent hydroxylation.

Methods. In the initial study, granulomas were induced by implanting weighed sterile gauze sponges (20 $\pm$ 1 mg) subcutaneously in the dorsum of 120-150 g male Sherman rats. Penicillin G (1500 units) was injected prophylactically. Five days after implantation, a group of animals was sacrificed; a second group was treated with the steroid to be tested (by direct injection into the sponge); and a third group remained untreated. On the ninth day the second and third groups of animals were sacrificed, and their sponges were removed. All of the sponges were weighed immediately after removal and again after drying to constant weight. The sponges were then macerated and extracted

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