Implantation of Functional Erythropoietic Elements Following Total-Body Irradiation.* (22082)

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(Introduced by A. Hollaender.)
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It has been demonstrated that recovery from lethal doses of X irradiation is promoted by the injection of living, nonirradiated bone marrow(1). It has been postulated that the introduced marrow cells proliferate and are responsible for recovery by cellular repopulation, or that they elaborate some humoral substance which stimulates recovery of the indigenous marrow. Transitory activity of the injected cells pending recovery of the irradiated marrow has also been suggested as the beneficial role played by the transfused marrow. It seems reasonable that marrow cells injected into an irradiated animal might proliferate and contribute cellular elements to the peripheral circulation of the host because of (a) the inhibiting effects of total-body irradiation on the immune response (2,3), (b) the demonstration of homologous transplantation into embryonic hosts(4-6) and of the postpartum tolerance by these hosts of skin transplants from the donor (7-10), and (c) the acquired tolerance by irradiated marrow-injected mice of skin grafts from the marrow donor(11). Demonstration of the establishment of a functional implant of foreign bone marrow in an irradiated animal would indicate that the amount of genetic difference which can be tolerated between the donor of a tissue transplant and the recipient may be increased by irradiation of the recipient, and would lend support to the cellular repopulation hypothesis as a factor in radiation recovery. Experiments with rats are described, in which marrow carrying an immunogenetic marker is utilized. It is shown that implanted marrow cells do contribute erythrocytes to the peripheral blood of irradiated animals for extended periods of time.

Experimental. Cellular antigens C and D in the rat are controlled by a single pair of alleles; erythrocytes of the 2 homozygotes, C/C and D/D, carry antigens C and D respectively, and those of the heterozygote, C/D, carry both antigens C and D(12). Cells of either homozygous type may be detected in a mixture of both by the specific agglutination of each with its homologous antiserum. Animals segregating for these genes as well as specific reagents against each antigen were kindly supplied by Dr. Ray D. Owen of the California Institute of Technology. Rats of one antigenic type were irradiated and subsequently injected with bone marrow of another type; the irradiation and injection schedules used in these experiments are described in Table I. Marrow was forced from the femora of freshly decapitated rats, suspended in Tyrode's solution, and immediately injected into the jugular vein of the anesthetized recipient. In the first experiment, the marrow from one femur was injected into each recipient, whereas marrow from two femora was generally used in Experiments 2 and 3. Unirradiated controls were similarly injected with the carrier. At various intervals following administration of the bone marrow, a drop of blood was collected from the tail of each surviving marrow-injected animal and suspended in citrate.

TABLE I. Schedule of Treatment of Rats Injected with Immunogenetically-Labeled Marrow.

Exp. No.	No. of animals	Dose (r)	Type of host	Type of implant	Time of marrow inj. post-irrad. (hr)
1a	10	900	C or D	CD	0– 3
b	10	900	CD	—	—
c	10	0	C or D	CD	0– 3
2a	11	750	C or D	CD	18–24
b	7	750	CD	—	—
3a	21	750	C or D	D or C	18–24
b	11	750	CD	—	—
c	7	0	C or D	D or C	18–24

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TABLE II. Results of Bone Marrow Administration following Total-Body Irradiation. Each day's results are represented by a fraction; numerator represents No. of animals showing in peripheral blood erythrocytes of implanted antigenic type, and denominator represents No. of surviving animals; on days when no blood typing was done, no numerator is present.

Exp. No.	Treatment	Time after irradiation (days) 0	6	8	10	14	18	20	22	28	35	46
1 a	X-ray + marrow	−/10	0/ 5	−/ 3	−/ 3	1/ 1	1/ 1	−/ 0				
b	X-ray + Tyrode's	−/10	−/ 8	−/ 0								
c	Marrow	−/10	−/10	−/10	−/10	0/10						
2 a	X-ray + marrow	−/11	−/11	0/ 4	−/ 4	3/ 3	3/ 3	3/ 3	2/ 2	2/ 2	2/ 2	1/1
b	X-ray + Tyrode's	−/ 7	−/ 7	−/ 6	−/ 6	−/ 5	−/ 5	−/ 5	−/ 4	−/ 4	−/ 3	−/3
3 a	X-ray + marrow	−/21	−/21	−/19	0/17	11/16	−/16	12/14	−/14	12/14	12/14	
b	X-ray + Tyrode's	−/11	−/11	−/10	−/ 8	−/ 5	−/ 5	−/ 5	−/ 4	−/ 4	−/ 4	
c	Marrow	−/ 7	−/ 7	−/ 7	0/ 7	0/ 7	−/ 7	0/ 7	−/ 7	0/ 7	0/ 7	

Each sample was washed once in 100 volumes of saline and diluted to a 2% cell suspension; this cell suspension was tested against anti-implant-type serum, against saline, and in some instances against antihost-type serum. Antisera were used in concentrations sufficient to effect complete agglutination of a 2% suspension of homologous erythrocytes. The mixtures were lightly centrifuged and examined for evidence of agglutination upon resuspension of the sedimented cells. The resuspended mixture was often examined microscopically to detect cell aggregates too small to discern with the unaided eye.

The *results* of the experiments are summarized in Table II. By the end of the second week, erythrocytes from the implant could be demonstrated circulating in the peripheral blood of most of the surviving irradiated, marrow-injected animals; the injected but unirradiated animals, on the other hand, showed no evidence of implanted cells at any time. The first indications of erythrocytes from the implant were generally visible only microscopically. A positive microscopic agglutination was usually followed within a few days by positive macroscopic agglutination. The length of time a marrow implant will persist is unknown; at the time of this communication, one mosaic animal from Exp. 2 is still living after 147 days, and 12 animals from Exp. 3 are still alive after 95 days with no evidence of implant regression.

The methods described by Wilkie and Becker(13) for quantitative determination of hemagglutinin titers were modified slightly and used to estimate the composition of the erythrocyte populations of the surviving rats in these experiments. First estimates on the thirty-seventh day postirradiation on 3 animals from Exp. 3 showed about 85% implant- and 15% host-type cells in two animals, and less than 5% implant- and 90% host-type cells in one animal. On the 65th and 95th days, postirradiation values for 12 animals ranged from less than 10% implant-type cells to close to 90%, the majority of the animals showing more than 60% implant-type cells. The sum of independent estimates of the percentage of host-type and implant-type cells was close to 100%, and agreement between estimates made on the 37th, 65th and 95th days was excellent. The sole survivor of Exp. 2 showed about 95% implant-type and 5% host-type cells on days 91, 112 and 147 postirradiation.

Discussion. The factors which determine the fate of tissue transplants have long been of great interest. It is known that the compatibility of a transplant is a function of the genetic similarity between the donor and the recipient; transplantation between identical twins or between individuals of a highly inbred strain is usually successful, whereas transplantation between less closely related individuals of the same species or between species is generally unsuccessful. There are, however, conditions under which successful transplantation can occur in the presence of greater genetic diversity between recipient and donor. If an embryonic animal is confronted with a foreign antigen throughout the development of its antibody-producing system, the postpartum reactions of the animal to the same antigen are profoundly affected. If the antigen is cellular, the foreign cells

may continue to divide and may actually differentiate and function in the host animal; this has been demonstrated for naturally administered erythroid elements in dizygotic twins in cattle(4,5) and in man(6) and for artificially administered erythroid elements in the rat [Ripley and Owen in(14)]. In all cases, the administered cells become established and produce mature erythrocytes giving rise to persistent erythrocyte mosaicism. Anderson *et al.*(10) have shown in cattle that mosaicism of one animal for erythrocytes of his twin renders that animal tolerant of skin grafts from the twin. Billingham *et al.*(8,9) have further shown that mice and chickens injected as embryos with homologous cells are more tolerant as adults of skin grafts from the donor of the injected cells.

It is probable that the reaction of an irradiated rat to a foreign cellular antigen is analogous to the reaction of an embryonic animal to the same cellular antigen. It has been shown by Dixon *et al.*(2) that the immune response of the rabbit can be temporarily eliminated by total-body irradiation in the dose range used in the experiments reported in this communication. It might be postulated that postirradiation administration of the cellular antigen results in continuous exposure of the reforming antibody-producing system to the foreign antigen; consequently, the reformed system is tolerant of a foreign antigen to which the original was not tolerant. Indeed, Main and Prehn(11) have shown that irradiated mice of one strain injected with bone marrow of a second strain are subsequently tolerant of skin grafts from the second strain; this observation, they conclude, is consistent with the hypothesis that the injected marrow elements proliferate.

Demonstration of persistent erythrocyte mosaicism in which (in some cases) more than 80% of the erythrocytes in the peripheral circulation have been derived from the implanted marrow makes it unnecessary to postulate a humoral factor or a transitory activity of the implant as the mechanism responsible for recovery of the erythropoietic and inferentially myelopoietic potential in irradiated rats treated with homologous marrow. The possibility remains that the introduced cells may produce, in irradiated animals, some substance which stimulates recovery and proliferation of marrow. It is recognized that an important contribution of erythropoietic recovery to general radiation recovery is not demonstrated in the data reported here, since no recovery-promoting effects of the marrow are evident (Table II). This observation is consistent with what is known of radiation death in rats, where the contribution of intestinal damage outweighs and precedes that of marrow damage(15).

An explanation tentatively hypothesized by Cole(16) to explain the radiation recovery brought about by the administration of living cells is that the injected cells repair genetic damage incurred by the irradiated cells by a process similar to bacterial transformation or transduction; this hypothesis is to explain his finding that nuclei and deoxyribonucleic acid extracts of spleen cells have recovery-promoting activity. On this hypothesis, it might be suggested that in the present case the cells of the implant antigenic type are descendants of a selected subpopulation of irradiated cells in which the damage has been repaired by transformation. Bacterial transformation occurs independently for unlinked loci(17,18). Complete transformation of a diploid cell from one homozygous type to another would consequently involve the intermediate formation of the heterozygous type, *i.e.*, CC$\rightarrow$CD$\rightarrow$DD, which was not detected. That the estimated proportions of CC and DD cells account for all erythrocytes is clearly incompatible with the concept of transformation at the chromosomal level.

Summary. Rats have been irradiated with doses of X-rays in the LD_{50} range and subsequently injected with homologous bone marrow carrying an immunogenetic marker. Erythrocytes carrying the label can be detected in the peripheral circulation of these animals within 2 to 3 weeks after administration of the marrow; unirradiated but marrow-injected controls do not show erythrocytes from the implant. Marrow implants have persisted and functioned for as long as 147 days at the time of this communication.

The implanted marrow contributes as much as 80% of the peripheral erythrocytes.

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A Simple Circulatory Bypass System.* (22083)

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Some method of total circulatory bypass is necessary to accomplish open intracardiac surgery. Ideally such a system should entail a minimum of danger to the subjects accompanied by a maximum of simplicity of working parts and operability. The use of homologous and heterologous lungs in combination with some type of mechanical pump has been shown by others(1,2) to most closely approach this ideal. This is a report of experimental work with an homologous lung system which has been further simplified.

Methods. Mongrel donor dogs weighing 10 to 20 kg were bled to death after receiving one mg of heparin per kilo of body weight. Heparin was then added to this blood in amounts of one mg per 100 cc of blood. This blood was used to prime the extracorporeal system, to replace blood loss, and to maintain the operated dog's blood pressure during surgery. No blood was given to any of the dogs after completion of the experimental surgical procedure. Following exsanguination of the donor dog, the left lung was removed and the bronchus and pulmonary artery were cannulated, the former being connected to a respirator and the latter to the discharge end of a 300-cc graduated cylinder. Experimental dogs weighing from 9 to 20 kg were then anesthetized with morphine and nembutal and the

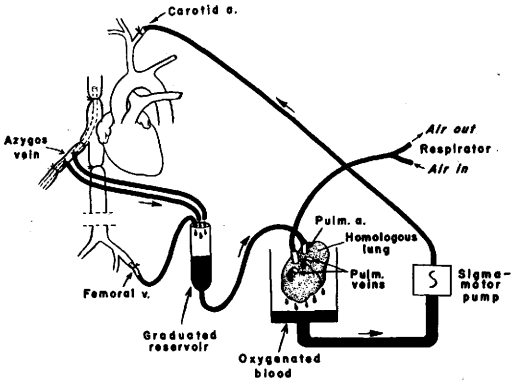

FIG. 1. Cannula connections of homologous lung blood oxygenator.

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