

MARROW TREATMENT OF IRRADIATED DOGS*

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TREATMENT with intravenous homologous bone marrow protects mice (Lorenz, Congdon and Uphoff, 1952), rats (Fishler, Cole, Bond and Milne, 1954), hamsters (Smith, Marston, Gonsbery, Alderman and Ruth, 1955), and rabbits (Porter and Murray, 1958) against the lethal effects of total body X-irradiation during the early post-irradiation period.

Similar treatment of irradiated dogs has not met with the same success (Rekers, Coulter and Warren, 1950). Use in the latter study of a dose of irradiation in the LD₅₀ range may have been a major factor contributing to failure, as it is now known that supralethal doses of radiation are usually necessary to obtain permanent marrow homotransplants (Trentin, 1956; Gengozian and Makinodan, 1957).

In the present experiments we have studied the influence of the dose of radiation on the success of marrow homotransplantation in the dog. Survival of the animal and temporary haemopoietic transplantation were seen in some dogs after 450 r and 500 r of X-rays. Larger doses of irradiation caused death in spite of evidence of haemopoietic repopulation.

MATERIALS AND METHODS

Male mongrel dogs weighing 7.0–9.4 kg. were used as recipient animals. After being anaesthetized with intravenous Nembutal they were exposed to whole body X-irradiation under the following conditions: 250 kv.p., 15 mA, 70 cm. target distance, 0.5 mm. Cu, 0.25 mm. Sn, and 1.0 mm. Al filters, HVL=3.0 mm. Cu, 30 r/min. dose rate. Calibration was carried out with a Victoreen ionization chamber.

The marrow for post-irradiation injection was obtained from 3-weeks–2-months-old female mongrel puppies. The donors were killed by an overdose of intravenous Nembutal and under sterile conditions the marrow from all 8 long limb bones was removed and gently agitated in cold physiological saline. The suspension was pressed through a nylon filter (porosity 90 μ) to exclude coarse bone spicules and then centrifuged at 600 r.p.m. for 5 min. to remove the fat. The residue was made up to 30 ml. by adding further saline and gently mixing. Nucleated cell counts were made on every sample and each dog received an average of 3250 ($\times 10^6$) cells (748–7000). The marrow suspension was given into a leg vein 1–3 hr. following irradiation. The control irradiated dogs were each given 30 ml. of saline intravenously.

After treatment all the dogs were fed milk, cooked minced meat and Purina dog chow. For the first 3 weeks they received in addition chlortetracycline hydrochloride (Aureomycin, Lederle) 250 mg. twice daily by mouth (Furth and Coulter, 1952).

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Total leucocyte counts and differentials, platelets, haemoglobin and microhaematocrits were determined every 2 days and neutrophils bearing nuclear "drumsticks" characteristic of the female (Porter, 1957a) were sought in stained films.

All animals dying were necropsied.

Experimental procedure

In this experiment a total of 37 dogs was studied, divided into 5 groups as indicated in the table. Group 1 were control animals given 450 r total body X-irradiation but no bone marrow (LD₅₀/30 days = 315 r). Groups 2, 3, 4 and 5 received 450 r, 500 r, 600 r and 700 r X-irradiation respectively followed by homologous bone marrow treatment.

TABLE.—*Effect of Homologous Bone Marrow on Dogs Exposed to Total Body X-irradiation*

Group	Number of dogs	X-ray dose (r)	Treatment	Number of dogs with marrow transplants	Number of dogs surviving 30 days	Mean survival time of those dying (days)
1	8	450	None	—	0	13.0
2	8	450	Bone marrow	1	1	12.5
3	8	500	" "	3	2	11.5
4	8	600	" "	4	0	9.9
5	5	700	" "	2	0	9.0

RESULTS

Dogs exposed to irradiation without marrow treatment

All animals in Group 1 (450 r ; no marrow) succumbed within 18 days.

Following total body irradiation there was a marked fall in the total leucocyte count which reached its lowest point at the 8th–14th day. Differential counts indicated that depression of the lymphocytes occurred more rapidly than depression of the neutrophils. The platelets fell a little more slowly, minimum values occurring from about the 12th day onwards. The haemoglobin and the packed cell volume declined gently until death.

Clinically, after a brief period of inactivity and malaise immediately after the irradiation, the dogs remained fit and ate well until about the 10th–12th day when they became listless, anorexic, pyrexial and developed bloody diarrhoea and respiratory difficulty.

Necropsy showed widespread purpura and occasional diffuse haemorrhages, the lymph nodes were enlarged and haemorrhagic, the tonsils ulcerated and there were haemorrhages into the mucosa of the gut from the stomach to the anus. In most there was non-purulent bronchopneumonic pulmonary consolidation ; in some there was evidence of generalized septicaemia.

Microscopically, although all the lymph nodes showed dilated sinuses filled with erythrocyte and haemosiderin laden phagocytes and clumps of red cells, lymphoid regeneration in the follicles and medullary cords was conspicuous. In all cases there was marked reduction in cellularity of the rib marrow, the venous sinuses were dilated and filled with red cells and occasional islands of active haemopoietic regeneration were present.

Irradiated dogs treated with bone marrow

Many of the marrow treated animals behaved in a similar fashion to those in Group 1 except that leucocyte depression occurred earlier, the mean survival

time diminished and gastro-intestinal damage became greater as the irradiation dose increased.

Ten animals, however, showed evidence of a successful marrow transplant. In these dogs (1 from Group 2, 3 from Group 3, 4 from Group 4 and 2 from Group 5) a rapid rise in the total leucocyte count began at 4-7 days and reached in those surviving about 6000 cells per c. mm. at 11 days (Fig.). At the same time typical female neutrophils appeared in the peripheral blood.

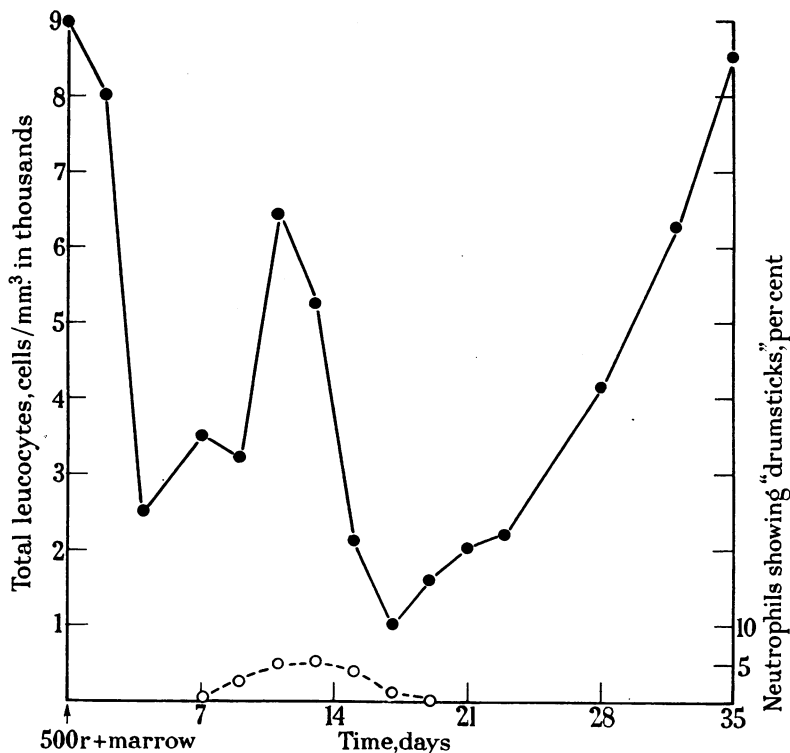


FIG.—Total leucocyte count and percentage of neutrophils showing female sex chromatin in X-irradiated male dog DR5 treated with homologous bone marrow and chlortetracycline.

●—● Total leucocytes.
○---○ Neutrophils showing "drumsticks".

Seven of the dogs with marrow transplants (those from Groups 4 and 5 and 1 from Group 3), died between 11 and 20 days after irradiation, still with evidence of haemopoietic recovery and female neutrophils. At necropsy there were indications of overwhelming terminal infection and extensive denudation of the intestinal mucosa. Haemorrhage was not a feature of these cases. The rib and vertebral marrow was hyperplastic and smears from some cases showed a few female cells. Colonization of the shafts of the long bones by islands of active haemopoietic cells had occurred in several of the dogs. Lymphoid regeneration was present as in the animals of Group 1. The lungs showed no infarcts, but in one animal (in the 500 r group) which died at 20 days a few tiny calcified deposits were found

within the lumina of small branches of the pulmonary veins. Some of these showed around their margins cells looking like osteoblasts.

The 3 survivors remained healthy, had good appetites and gained weight, but by the 14th day their white cell counts had fallen to about 3000 cells, and this sharp decrease continued to about 1000 cells on days 16-18. Simultaneously, the percentage of neutrophils showing female characteristics diminished until about the 20th day when all had disappeared. From about the 18th-19th day the total white cell count in the 3 dogs rose slowly again reaching 9000 per c. mm. at about the 35th day, but female neutrophils were no longer to be found.

DISCUSSION

In these experiments transplantation of bone marrow between genetically dissimilar mongrel dogs has been achieved following doses of whole body X-irradiation well above the LD₅₀/30 day level for these animals (315 r). But many of the dogs with homologous transplants died. Death in the majority occurred early apparently from gross fluid and salt loss from the gut with a superimposed terminal infection. This heavy mortality from an intestinal syndrome, despite commencing haemopoietic recovery from a successful marrow transplant, also occurs in the rat (Fishler *et al.*, 1954) and the rabbit (Porter, 1957b). In the 3 dogs which survived there was evidence that the marrow homotransplant was temporary, in that donor type female neutrophils appeared as haemopoietic recovery occurred, but disappeared again later. Presumably the doses of radiation used (450 r and 500 r) briefly suppressed the immune mechanism sufficiently to allow the foreign cells to colonize the depleted host marrow spaces, but when the recipient's ability to respond returned, the transplant was destroyed. Such host rejection of the graft is often seen after sublethal irradiation and may result in a higher mortality in the marrow treated animals than in those exposed to irradiation alone (Genzozian and Makinodan, 1957).

Treatment with electrolytes, plasma and protein hydrolysates have prolonged the survival of dogs exposed to as much as 1700 r (Conard, Cronkite, Brecher and Strome, 1956). Such treatment applied to our 600 r and 700 r groups might well have reduced the early mortality and allowed survival since haemopoietic recovery from transplanted marrow was occurring in such animals. To have given the irradiation in divided dosage might also have been an advantage as has been shown in the rabbit (Porter and Murray, 1958). Recently a successful marrow homotransplant has been obtained in a dog following 1200 r given in doses of 400 r on each of 3 successive days (Ferrebee, Lochte, Jaretzk, Sahler and Thomas, 1958). The total leucocyte count in the animal described by these authors fell after initial recovery in a way identical to that seen in our dogs, but whereas in our cases female neutrophils never reappeared, in Ferrebee's case it seems they were again found at the 40th day, even though in an earlier report on this dog (Ferrebee, 1958) it was stated that "the next rise in W.B.C. was due to proliferation of this dog's own leucocytes and not that of the transplanted marrow".

The bone marrow used in our studies was obtained from puppies largely to simplify technique as it is easy to scrape large quantities of active marrow from the shafts of the long bones of young animals. In adult dogs these bones contain mostly fat. In future work it would seem wise, however, to use foetal donor tissue in an attempt to avoid any possible late immunological reactions of transplanted tissue against host antigens (Uphoff, 1958).

In Reker's (1950) original experiments multiple bone spicules were found in the smaller pulmonary veins, and in one of our dogs a few similar calcified deposits were found. It is not possible at present to say if this is likely to be a common complication in higher animals of marrow treatment by the intravenous route. In rodents a pencil of marrow relatively free from bone spicules can be obtained with ease from the shaft of any long bone. Similar preparations from dogs inevitably contain many tiny bone fragments and osteoblasts. Filters of finer porosity than the 90 μ nylon mesh employed in our study should perhaps in future be used.

It was hoped by the use of chlortetracycline hydrochloride to reduce the incidence of overwhelming post-irradiation bacterial invasion, but like Furth, Coulter, Miller, Howland and Swisher (1953) we found little evidence of benefit.

SUMMARY

Bone marrow transplantation between mongrel dogs was attempted after several different doses of X-irradiation.

Temporary transplants of donor marrow and survival of the recipient were observed in 12.5 per cent of the animals after 450 r and in 37.5 per cent after 500 r (LD₅₀/30 days 315 r).

Although there was evidence of an initially successful marrow homograft in 50 per cent of the dogs exposed to 600 r and 40 per cent of those receiving 700 r, early death from intestinal damage occurred in all.

No permanent marrow transplants were achieved.

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