

Comparative Effectiveness of Fresh and Lyophilized Platelets in Controlling Irradiation Hemorrhage in the Rat.* (24481)

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It has been established that thrombocytopenia is the major cause of hemorrhage in animals exposed to uniform whole body irradiation(1). Repetitive transfusions of fresh platelets, to maintain reasonable platelet levels, prevent hemorrhage. Bleeding that has commenced can be quantitatively suppressed by a single fresh platelet transfusion (2). The storage life of platelets is measured in hours. Accordingly, considerable efforts have been exerted to preserve platelets by freezing(3), storage in media(4), or by lyophilization(5). Platelets preserved by the preceding 3 methods have been reported of value in controlling bleeding in leukemic children(5). However, the methods of assaying effectiveness of platelet transfusions in patients are not sufficiently quantitative. Since radiation injury may occur accidentally in the atomic industry and would be seen on a large scale in nuclear warfare, and intermittently after chemotherapy of cancer, it appeared essential to evaluate the effectiveness of preserved platelets in controlled radiation injury by quantitative methods. This report deals with the comparative effectiveness of fresh and lyophilized platelets in controlling hemorrhage in fatally irradiated rats.

Methods and materials. Sprague-Dawley rats were used, females and males approximately 100 g at time of irradiation. Donor rats were cast off female breeders about 300 g in weight. Platelet counts were performed by Brecher-Cronkite phase contrast method(6). Rats were given 600 r total body irradiation by 250 KVP General Electric x-ray machine operating at 30 ma and filtered with 0.5 mm of Cu and 1 mm of Al. Tissue dose rate was 32 r/minute at TSD of 100 cm. Donors were bled from abdominal aorta under sterile con-

ditions into cold siliconed syringes containing 1 ml of cold 1% Na₂ EDTA in 0.7% saline. The blood was then placed in cold siliconed tubes and platelets were separated by modification of the differential centrifugation technique of Dillard *et al.*(7). Freshly separated platelets were either transfused within 2 hours or lyophilized precisely according to the method of Klein, *et al.*(5) and stored in sealed ampoules filled with dry nitrogen. In a preliminary experiment to evaluate histologically the effectiveness of platelets in preventing hemorrhage, 18 rats were given 600 r. Three groups of 6 animals each were to have received transfusions of fresh platelets, lyophilized platelets and saline, respectively, on days 6, 8 and 10, with sacrifice to follow on day 11. The amount of fresh platelets transfused was estimated to raise platelet count to between 1 and 2 x 10⁶ platelets/mm³. Equivalent amounts of lyophilized platelet material were given. All animals were transfused on 6th and 8th day. At time of second transfusion on 8th day, one animal receiving lyophilized platelets died within minutes in convulsion. Then one animal from each of the other groups was sacrificed. Similarly on day 10, 2 animals receiving lyophilized platelets died, and 2 from the other groups were sacrificed. The remaining animals were sacrificed on day 11. At sacrifice, tissues were prepared for histological examination. In a second series of experiments, fresh platelets in amounts indicated above, were given on 5th, 7th and 9th post-irradiation days to 5 rats, and lyophilized platelets to 5 rats. All animals receiving transfusions of fresh or lyophilized platelets were sacrificed on day 10. Eight animals were subjected to irradiation only, and 2 were sacrificed on each of post-irradiation days 3, 5, 7 and 10. Four control animals receiving no treatment were sacrificed on day 10. All tissues were prepared for histological examination. In Exp. 2, one animal died

* Research supported by U. S. Atomic Energy Comm.

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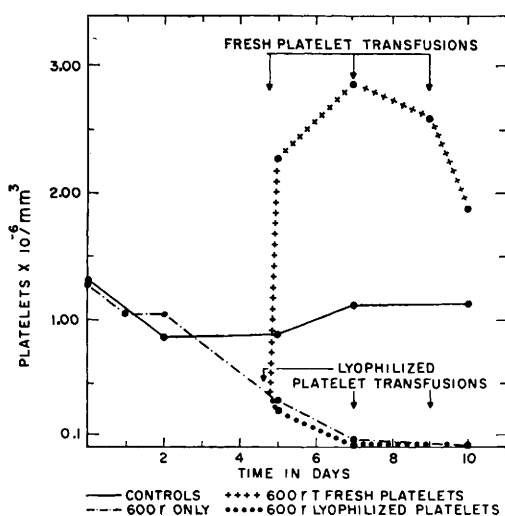


FIG. 1. Effect of transfusions of fresh and lyophilized platelets on platelet count following exposure to total body irradiation in the rat.

shortly after receiving a transfusion of lyophilized platelets on day 9, and was not included in data given above. Several tests were used to assay for presence of bleeding and the effectiveness of transfusions as follows: 1. Gross observation for bleeding. 2. Platelet counts. 3. Study of hematocrit. 4. Observation for and microscopic evidence of bleeding and concentration of red cells in lymph nodes. 5. Susceptibility to bleeding from minor trauma (subcutaneous injection of 0.5 ml of isotonic saline).

Results. Fig. 1 presents comparative platelet counts of control non-irradiated rats, control irradiated rats given no transfusions, rats irradiated and given fresh platelet transfusions, and rats irradiated and given lyophilized platelet transfusions. Fresh platelet transfusions raised and maintained the platelet count at more than twice the normal level. There was no detectable difference in platelet count between non-transfused irradiated rats and irradiated rats receiving lyophilized platelet transfusions.

Fig. 2 shows comparative serial hematocrits for all groups of rats. The constancy of the hematocrit in non-irradiated rats demonstrates that blood sampling did not affect the hematocrit. Irradiated non-transfused rats developed the usual anemia expected on the basis of past experience. The decrease in hema-

tocrit of rats receiving platelet transfusions was considerably less rapid than in control irradiated or lyophilized transfused irradiated rats. Rats receiving lyophilized platelets developed a more severe anemia than control irradiated rats.

At autopsy on eighth to eleventh post-irradiation day, diffusely bloody lymph nodes were seen grossly and microscopically in control-irradiated and lyophilized-platelet-transfused groups. Control non-irradiated and fresh platelet transfused rats showed no gross hemorrhage, and only a rare extravascular red cell microscopically in lymph nodes of the platelet transfused group. In all groups, phagocytized red cells were found in the lymph nodes.

At sites of saline injection, ecchymoses were seen in the control-irradiated and lyophilized-transfused groups. No ecchymoses were seen in control non-irradiated or in irradiated fresh platelet transfused groups.

Fresh platelet transfusions were tolerated without visible distress. The lyophilized platelet transfusions regularly produced vessel spasm at site of injection. Frequently, respiratory stress and hyperexcitability followed injection of lyophilized platelets. This passed off promptly in most instances, although several deaths resulted as noted above.

Discussion. In the irradiated rat, fresh platelets prevented bleeding completely. Lyophilized platelets in similar amounts were totally ineffective. In the fresh platelet trans-

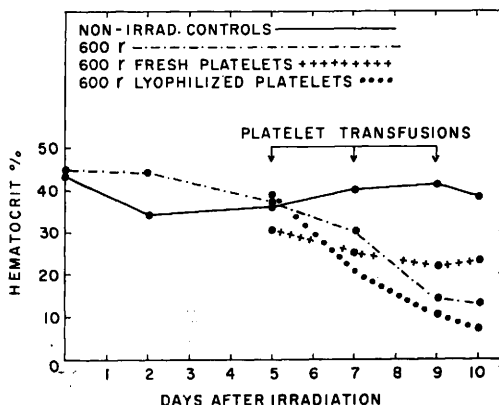


FIG. 2. Effect of transfusions of fresh and lyophilized platelets on hematocrit following exposure to total body irradiation in the rat.

fused group, since there was no hemorrhage, the decrease in hematocrit was primarily due to cessation in new cell production and senescence of red cells present at time of irradiation. If total blood volume remained constant, the red cell mass decrease, as indicated by the hematocrit, would have been about 70% over a 10-day period. Thus a very rough estimate of rat red cell life span by this manner is in excess of 20 days.

It could be argued that larger amounts of lyophilized platelets might be needed to control bleeding. However, the amounts injected were at a level resulting in acute mortality in some animals.

These observations do not apply to frozen platelets and do not prove that lyophilized platelets are of no value in other bleeding states. Since radiation bleeding apparently is due almost exclusively to a simple thrombopenia one can conclude that lyophilized platelets are ineffective in controlling bleeding from uncomplicated thrombocytopenia, whereas fresh platelets are effective in controlling such bleeding.

Summary. The efficiency of fresh *vs.* lyophilized platelet transfusions to control radiation induced hemorrhage were tested in rats by gross observation for bleeding, platelet count, hematocrit, microscopical study of

lymph node sections, and susceptibility to bleeding from minor trauma. Hemorrhage occurs in irradiated, untreated rats around the fifth post irradiation day increasing with the progress of platelet fall, which reach a minimum around the tenth day. Fresh and lyophilized platelet transfusions were performed on 5th, 7th and 9th day. Whereas fresh platelet transfusions raised the platelet count to 2-3 times the normal and stopped hemorrhage, lyophilized material had no effect on platelet counts which were not different from irradiated controls and did not show any influence on the bleeding tendency. In the lyophilized platelet transfused rats, the hematocrit dropped more than in the irradiated controls.

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Received September 15, 1958. P.S.E.B.M., 1958, v99.

Total Body Water in Growing Domestic Fowl by Antipyrine Dilution Technic. (24482)

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That total body water was nearly a direct proportion of fat-free weight was shown by Light *et al.*(1) to be true for any particular age group but not between ages, in weanling and in grown rats. Annegers(2) studied the relationship among total body water, body fat, and fat-free, dry body weight in normal rats and mice and in starved and dehydrated

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rats. He showed that body fat did not have an independent effect on total body water when expressed on a fat-free basis. Severe dehydration reduced total body water in adult female rats. Distribution of non-toxic analgesic, antipyrine, in tissues in proportion to their water content was observed by Brodie and Axelrod(3). The volumes of distribution of antipyrine and deuterium oxide were found to agree well in normal subjects. Good agreement has also been obtained when com-