Control of the Postirradiation Hemorrhagic State by Platelet Transfusions

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The hemorrhagic state following massive irradiation is characterized by the appearance of erythrocytes in large numbers in the lymph. The peak of this erythrocyte diversion into the lymph is reached in rats at nine to fourteen days and in dogs at eleven to seventeen days after irradiation, at which time the erythrocyte counts in the lymph often exceed one million per cu. mm. A relationship between irradiation thrombocytopenia, first observed by Lacassagne and Lavedan, amid bleeding into the tissues was clearly indicated by Shouse et al. The parallel between the postirradiation hemorrhagic tendency and the decrease in circulating platelets has been confirmed by other workers, and the concept of “hyperheparinemia” as a major cause of radiation hemorrhage has been abandoned. A new impetus to studies of the role of platelet deficiency in radiation injury was given by the work of Cronkite et al., who demonstrated that daily injections of platelet suspensions begun before thrombocytopenia developed prevented the hemorrhagic phase as seen at autopsy.

This study describes the extent to which single platelet injections diminish the hemorrhagic tendency of irradiated dogs and rats as indicated by erythrocyte counts in the lymph. In the experiments of Cronkite et al., irradiated platelet-transfused animals were contrasted with untreated animals, and thus only a clinical comparison of the platelet effect was possible. The lymph fistula animals used in this study, however, served to a large extent as their own controls, and the platelet effect could thereby be expressed quantitatively with respect to the degree of the hemorrhagic state before treatment and improvement in relation to time after treatment.

Material and Methods

Surgical Preparations

Exteriorized anastomoses between the thoracic duct at the left supraclavicular region and a branch of the external jugular vein were prepared in dogs as described by Ross et al. The mesenteric lymph fistulas in rats were prepared by the method of Bollman et al.

Experimental Animals

Healthy, adult, mongrel, male dogs weighing between 17 and 27 pounds were used for irradiation, cannulation and transfusion of platelet suspensions. Those used as platelet donors weighed 45 to 65 pounds. The dogs were vaccinated for distemper and rabies, and were dewormed, with 2 exceptions. White blood cell counts were made routinely before and

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after x-irradiation. The animals to receive platelets were x-rayed with 350 to 450 r, equivalent to between LD50 and LD90, thirty days. A white blood cell count of less than 1500 at four to six days after irradiation was taken as an indication that the exposure was biologically effective. The irradiated dogs received 400,000 units per day of procaine penicillin G intramuscularly from the time of irradiation until death. Rectal temperatures of the irradiated animals were recorded daily at 9 a.m. Commercial dog food was supplied at 4 p.m., and water was allowed ad libitum.

The rats* used were three to four month old males weighing between 200 and 250 Gm. They were observed for apparent freedom from disease for two weeks before use. Selection as experimental pairs (platelet-injected animal and plasma-injected control) was made on the basis of approximate identity of age, sex, weight, blood-platelet levels, x-ray exposure and time of cannulation. The rats to be cannulated received 600 to 750 r of x rays, equivalent to between LD50 and LD90, thirty days. A blood platelet level of 30,000 or less at eight to nine days after irradiation was arbitrarily taken as an indication that the animals were in the hemorrhagic phase and satisfactory for the study of the effect of platelet transfusion. Antibiotics were not given to the rats. A good commercial stock diet and water were supplied ad libitum to all animals. Healthy rats from the same stock were used as donors for the platelet preparations.

Factors of Irradiation

Dogs: 250 kvp, 30 ma., 3 mm. Al, TSD 117 cm., average proximal skin dose 49 r/min. The dogs were anesthetized with Nembutal (25 mg/Kg.) and were turned over at half-time exposure.

Rats: 250 kvp, 30 ma., 3 mm. Al, TSD 93 cm., average proximal skin dose 75 r/min. The animals were unanesthetized and their positions were not altered during exposure.

The x-ray dose was monitored with a 100 r Victoreen chamber.

Platelet Preparation and Transfusion

The preparation of dog platelets for transfusion was made according to the method of Dillard et al. The blood was collected by gravity flow into siliconed centrifuge bottles contained in an ice bath. All collections were made directly into a 1 per cent solution of disodium Sequestrene (ethylene diamine tetraacetate) in 0.7 per cent sodium chloride, one volume of anticoagulant solution being used for nine volumes of blood. Platelets prepared from the blood of 2 donors were ordinarily sufficient to raise the platelet level of an irradiated, lymph-cannulated recipient above 100,000.

Lymph-venous anastomoses were established in dogs seven to nine days after x-ray exposure. When the blood platelet and lymph erythrocyte levels indicated a suitable hemorrhagic state, the dogs were anesthetized with Nembutal and the platelet suspension in fresh plasma was transfused through the venous cannula. The suspension was flushed through the cannula with 0.9 per cent saline or 10 per cent glucose in saline. The platelet suspension was injected within four hours after withdrawal of the blood from which it was prepared. During this period the platelets were kept at 2 to 3 C.

Suspensions of rat platelets were prepared by the same method. From rats under Nembutal anesthesia (40 mg/Kg.), heart blood was drawn into a siliconed 10 cc. syringe containing 1 cc. of Sequestrene solution. Withdrawal of blood equivalent to 3 per cent of the body weight usually was not fatal and permitted repeated bleedings of a donor. After collection, the blood was quickly transferred to graduated, siliconed centrifuge tubes maintained in an ice bath.

In rats with platelet levels below 35,000, mesenteric lymph fistulas were prepared eight to eleven days after irradiation, and the lymph erythrocyte counts were followed for 4 to 6 hours. Injections of platelets were made through a surgically exposed external jugular vein.

* Obtained from the Wistar Institute, Philadelphia, Pa.
† Glassware was siliconed with “Desicote,” Beckman Instrument Company, Pasadena, Calif.
‡ Alrose Chemical Company, Providence, R. I.
with the animal under Nembutal anesthesia. Blood from 1 to 2 donors was used to provide platelets for each recipient.

**Platelet Counts**

The platelet counts were made as described by Brecher and Cronkite.14 In dogs, the blood was obtained from a superficial vein, with free flow from a 21 gauge hypodermic needle. The rats were anesthetized with ether, the skin over a femoral vein shaved and rubbed with a silicone stopcock grease, the vein punctured and blood taken from a freely bleeding wound. The platelets were counted under the phase microscope at 1:100 dilution with 1 per cent ammonium oxalate.

**Erythrocyte Content and Flow of Lymph**

To minimize the effects of operative trauma, examination of the lymph before platelet injection was extended over 24 to 48 hours in dogs, and 4 to 6 hours in rats. Cell counts were made from lymph taken into a counting pipet directly from the freely flowing lymph cannulas and diluted with Toison’s solution. In dogs, the white cell count was determined by direct count and also estimated by differential counts of smears. In rats, the white cell count was estimated only by the latter procedure.

After injection of the platelets, the cellular content of the lymph was determined frequently during the first 6 hours, then at intervals of 6 to 24 hours until termination of the experiment. In the dogs the lymph-venous anastomoses were re-established between samplings, while the rat lymph was permitted to drain continuously. At the time of lymph sampling, the dogs were immobilized and the rate of lymph flow measured until a constant rate was established, usually after 30 minutes of free flow. This procedure minimized false erythrocyte counts due to obstruction of the cannula. Following platelet transfusion, progressive thrombus formation occurred around the tip of the venous cannula and within 36 to 72 hours the return flow of lymph was completely blocked. Erythrocyte counts in the lymph and the blood platelet levels were followed until the death of the dog, or until the lymph-venous anastomoses became occluded, at which time the animals were killed.

The lymph flow was recorded in the cannulated rats throughout the period of observation, a free flow being required for reliable cell counts. Thin clots were removed and the lymph flow was allowed to flush out the cannula before sampling.

**Results**

Suspensions containing 9.0 to 22.8 × 10^10 platelets were injected in a plasma volume of 50 to 100 cc. in 6 dogs with blood platelet levels of 30,000 or less and lymph erythrocytes of 6.5 to 90 × 10^9. The results are shown in figure 1. The immediate rise in blood platelet levels and their retention in the circulation for 36 to 48 hours may be taken to indicate the excellent preservation of most of the platelets in the course of preparation. The observed platelet levels at 15 to 30 minutes after transfusion were from 55 to 95 per cent of the calculated possible rise. The profound depression of lymph erythrocyte counts was almost complete within 5 to 6 hours after platelet injection, and remained low until the blood platelet level fell to near the preinjection level, at which point the bleeding began again. During the period of observation, the circulating platelet level decreased at a rate of approximately 3000 platelets per cu. mm. per hour shortly after injection. This rate declined to approximately 600 per hour at 20 to 48 hours.

The curve of increase and duration of appearance of erythrocytes in the lymph of dogs given similar doses has been previously established.2 A slow seepage of erythrocytes into the

* Based on an assumed blood volume of 8 per cent of body weight.
lymph begins after three to five days followed invariably by a rapid rise during the next two to four days to levels of about one million. In 1 control animal 70 cc. of plasma caused no depression of the erythrocyte content of the lymph. Because of post-mortem evidence of partial thoracic duct obstruction, the erythrocyte counts in the lymph of dog 4 are not included in figure 1.

The results of platelet injections in 5 rats at nine to twelve days after irradiation are shown in figure 2. Suspensions of $1.6 \times 10^6$ to $4.3 \times 10^6$ platelets in 0.35 to 0.50 cc. raised the circulating platelet levels of the recipients from a preinjection level of 50,000 or less by 70 to 92 per cent of the calculated rise* at 15 to 30 minutes after injection. These rats lost circulating platelets at a rate averaging 5000 per hour for more than 18 hours after injection. In the 5 matched controls receiving an equal volume of the plasma in which the platelets were suspended, the platelet level remained below 50,000.

Blood platelet and lymph erythrocyte levels were followed in four pairs for 18 hours after injection of platelets and plasma respectively, when both experimental animal and control were sacrificed for autopsy. In a fifth platelet-injected rat the preinjection lymph erythro-

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* Based on an assumed blood volume of 6 per cent of body weight.\(^5\)
The rapidity with which the platelet infusion corrected the capillary hemorrhage is remarkable. The bloody lymph became clearer consistently during the first 30 minutes to 1 hour after platelet transfusion, and in one rat as early as 20 minutes after injection. Due to manipulation of the rats during anesthesia and injection of the platelets, trauma may have modified the lymph erythrocyte counts during the first 2 hours after injection, raising the erythrocyte levels which were depressed by the platelet transfusion. Depression of erythrocyte levels in the lymph was, however, almost complete within 5 to 6 hours. When the hemorrhage was more severe, a longer time and a greater quantity of platelets was required to control it. The slower rate of decrease in erythrocyte levels in the lymph
of the more hemorrhagic animals may be due in part to a longer time required to free the lymphatic system of the erythrocytes which had flooded it before the administration of platelets.

The critical platelet level at which hemorrhage began, as manifested by the appearance of large numbers of erythrocytes in the lymph, appears to be between 25,000 to 50,000 per cu. mm. in both dogs and rats. This is close to the well known hemorrhagic threshold level of approximately 60,000 in man. Experience in the selection of suitable hemorrhagic animals in this study suggests that the lymph will become grossly bloody within 24 hours after a platelet drop below approximately 35,000.

At autopsy all dogs showed changes characteristic of acute radiation injury with hemorrhages. This does not contradict the findings of Cronkite, et al. who injected platelets repeatedly before or after thrombocytopenia developed and found few hemorrhages at death. In the present experiments, bleeding varied from punctate to massive hemorrhages. Reduction in the usual bleeding tendency was, however, discernible. Intestinal hemorrhages were absent or few. The lymph nodes were yellow-brown (due to hemosiderosis) or slightly pink in 4 dogs and characteristically intensely red in the other 2 dogs. In earlier experiments the lymph nodes of all untreated dogs similarly irradiated which died during the hemorrhagic phase were intensely red. The decrease in the hemorrhagic tendency in lymphatics and lymph nodes draining different body areas was not uniform, e.g., the hepatic lymphatics in 1 dog were intensely red while the other lymphatics were only yellow-gray. In the lungs, petechiae were few in 3 dogs while the others had the usual marked extensive hemorrhages. Two dogs had gross manifestations of gas bacillus infection. All dogs had terminal hyperpyrexia, the temperatures ranging between 104 and 106 F. for 24 to 72 hours preceding death.

In four experiments, a direct comparison was made of the platelet-injected rats with their plasma-injected controls. Hemorrhages in the platelet recipients were generally less severe, especially within the lymphatic system, notably in the mesenteric lymph nodes. However in 2 rats there were more petechiae in the lungs of the controls than in those receiving platelets. No difference in the degree of intestinal hemorrhage was evident between controls and platelet-injected rats.

**Discussion**

The data presented indicate that the passage of erythrocytes into the lymph of dogs and rats after exposure to massive doses of x-rays is dependent on the blood platelet level, and support the findings of Cronkite et al. that transfusions of platelets control this hemorrhagic phase. The studies presented here show the quantitative correlation between blood platelet and lymph erythrocyte levels and indicate that the hemorrhagic state can be reversed even when well established.

Clinical manifestations of hemorrhage are no dependable index of the underlying process, namely diversion of erythrocytes from blood to lymph spaces. Only if the latter is advanced are clinical signs of significance, and even then they need not be conspicuous. Thus, some apparent discrepancies become clarified; e.g., Duruing et al. noted a drop in the platelet count of a dog to 10,000 three weeks after exposure to 775 r in 31 exposures of 25 r each, but found no clinical evidence of spontaneous hemorrhage. In the experiments herein described, using erythrocyte diversion as an index, hemorrhage in varying degree was invariable within 24 hours after the platelet count had been below 35,000.

The mode of action of platelets in ameliorating radiation hemorrhage is not
indicated by the present studies. The platelet is postulated to participate in the clotting reaction in the formation of thrombin, to promote hemostasis mechanically by forming platelet thrombi, and to yield a vasoconstrictor substance.\textsuperscript{19} Furthermore, it is possible that the platelet exercises a role in preventing or repairing endothelial damage in a way still not understood. It is noteworthy that at least three research groups have independently suggested that the platelet materially contributes to the actual formation of inter-endothelial "cement substance" and that lack of the latter is the major cause of the hemorrhagic state accompanying thrombocytopenia.\textsuperscript{2, s, o, 29} That actions other than those involved in the clotting reaction may be operative here is indicated by the findings\textsuperscript{8, 12} that a blood level of 100,000 platelets was required in vivo to correct the coagulation defects (whole blood clotting time and prothrombin utilization) of massively irradiated dogs, while bleeding was greatly diminished by raising platelet counts to lower levels well below 100,000.

Extensive efforts were made\textsuperscript{31} to find a sub-cellular fraction which will duplicate the effect of intact platelets in irradiated rats using the sensitive technic of measuring lymph erythrocyte levels. Platelet suspensions fragmented by ultrasonic or supersonic vibration or by alternate freezing and thawing,\textsuperscript{6, 22} or lyophilized and reconstituted with saline prior to injection, proved to be biologically ineffective. Following the idea that the main role of the platelet is that of a mobile thromboplastin carrier, fresh rat-brain thromboplastin was administered to rats in massive doses intraperitoneally; this likewise failed to depress the erythrocyte counts in the lymph of irradiated rats. Rutin administered intravenously or intraperitoneally had no effect in depressing the lymph erythrocyte counts.

The anemia of irradiation has several factors: depression of erythropoietic centers, diversion of erythrocytes into lymph spaces and destruction of erythrocytes.\textsuperscript{6, 23} Platelet transfusion will not correct the first, but will certainly correct the second and perhaps some of the third factor, which may be in part the consequence of the second.\textsuperscript{23} Since the depression of the erythrocyte mass resulting from complete lack of erythropoiesis amounts to only about 1 per cent per day, even the anemia during the second and third postirradiation weeks will not reach critical levels if the hemorrhagic state is corrected. Platelet transfusions might reduce the severity of the anemia below the critical level. The therapeutic possibilities of transfusions of platelets in other diseases involving vascular alteration or thrombocytopenia are obvious.

**Summary**

Suspensions of concentrated, homologous blood platelets when introduced intravenously into lymph-cannulated dogs and rats (nine to twelve days after exposure to an LD\textsubscript{50} or greater dose of x-rays) have been shown to eliminate the large scale diversion of erythrocytes into the lymph during the hemorrhagic phase of radiation injury. By means of injection of platelets preinjection levels of below 30,000 were raised to an average of 107,000 in 6 dogs, and to an average of 310,000 in five rats at 15 to 30 minutes after injection. All platelet-injected animals showed clearing of the bloody lymph within the first hour after injection, and within 5 to 6 hours the average erythrocyte count in the dogs dropped from an average of 270,000 to an average of 12,000 and in the rats from an average of
900,000 to an average of 30,000. No depression of lymph erythrocyte counts was found in control rats receiving plasma only. Lymph erythrocyte counts in the platelet recipients remained low for 24 to 36 hours after injection; bleeding into the lymph space reappeared in both rats and dogs when the platelet level dropped below approximately 50,000. Blood platelet and lymph erythrocyte levels are excellent indices to quantitate the severity of the hemorrhagic phase of radiation injury and to gauge the results of therapeutic procedures.

While the mechanism of platelet action remains to be demonstrated, the present studies support the thesis of earlier workers that platelets are essential to maintain vascular integrity and that a platelet deficiency is the main cause of the post-irradiation hemorrhagic state.

**Conclusions**

1. The appearance of large numbers of erythrocytes in the lymph of dogs and rats exposed to massive doses of ionizing radiation is partially or completely prevented by transfusion of concentrated suspensions of intact, homologous blood platelets.

2. The quantitative correlation of the blood platelet and lymph erythrocyte levels is a more sensitive index of the hemorrhagic state than the anatomic changes seen at autopsy.

3. Hemorrhage occurs in dogs and rats after the platelet level of the blood drops below 50,000 per cu. mm.

4. Passage of erythrocytes from the blood to lymph is retarded almost immediately after transfusion and the lymph erythrocyte levels are markedly reduced.

**References**


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