*Original Article*

# Cellular and Biochemical Effects of Combined X-Ray Radiation and Storage on Whole Blood

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## Abstract

**Background:** Evaluating the impact of ionizing radiation on stored blood is relevant since blood banks are major assets in emergency conditions such as radiation incident/attack. This study aimed to fill our knowledge gap of combined radiation and storage effects on blood.

**Methods:** Blood collected from 16 anesthetized rats was anticoagulated, aliquoted into storage bags, and assigned to 8 groups using protocols combining storage (1-day vs 3-day 4°C) plus irradiation (75 Gy vs 0 Gy - control). Bags were positioned inside an X-ray irradiator (MultiRad-350). Complete blood count, differential white blood cell count, biochemistry, and hemostasis were analyzed (≥7 bags/group).

**Results:** $Na^+$, bicarbonate, glucose, and pH significantly reduced, while $K^+$, $Cl^-$, and lactate increased by storage. Coagulation measures were not significantly altered after radiation. White blood cell count and most cell types were numerically reduced after radiation, but changes were statistically significant only for monocytes. No significant alterations were noted in aggregation or rotational thromboelastometry parameters between irradiated and control.

**Conclusions:** Evaluating cellular/biochemical parameters aids in assessing stored blood adequacy after radiation. Data suggest that fresh or cold-stored blood can sustain up to 75 Gy without major critical parameter changes and may remain suitable for use in critically ill patients in military/civilian settings.

## Keywords

X-ray radiation, cold storage, platelets, storage lesion, cell damage, resuscitation, rat, whole blood, ionizing radiation, aggregometry

## Introduction

Humans may be exposed to ionizing radiation from natural sources such as cosmic rays, radioisotopes, and multiple artificial sources. Exposure to high-dose radiation may not be very common, but biological damage induced by ionizing radiation can lead to serious effects, such as gene mutation, cellular senescence, necrosis and/or apoptosis, and cell death.[1]

However, the effects of radiation on blood, especially on stored blood, are not well understood and scarcely studied. As an efficacious intervention for critically ill patients in military and civilian settings, whole blood (WB) is being used with increased frequency and has become the preferred product for resuscitation of severe traumatic hemorrhage.[2] Cold-stored WB offers the benefit of a balanced resuscitation with improved survival in addition to higher hemoglobin levels.[3]

Nevertheless, blood storage results in biochemical, structural, and metabolic changes, referred to as storage lesion, that

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may mediate adverse effects associated with transfusion of older red blood cell (RBC) units. These include increased hemolysis, oxidative stress, and accelerated scavenging of nitric oxide. Somewhat similar changes occur to other cells in stored WB.[4]

X-rays are widely used for security inspection, X-ray fluorescence, and nondestructive testing imaging. X-ray irradiators and gamma irradiators produce a wide range of ionizing radiation doses, and some studies have shown that they provide equivalent effects for in vitro and in vivo applications. However, X-ray irradiators are being increasingly used in clinical settings to replace gamma ray irradiators, due to their low cost and absence of radioactive source.[5]

An accidental exposure or terrorist event may cause specific biological tissues such as stored blood to be also exposed to higher levels of radiation.[1,6] It is possible to experimentally study exposure to high levels of radiation using self-contained X-ray irradiation systems.[7] These systems produce typical ionizing radiation levels that members of the public could undergo medical diagnosis, radiotherapy, occupational exposures, or even in a radiological disaster. Their versatility allows controlled irradiation studies of cultured cells, blood storage bags, and experimental animals.[8]

This article focuses on important but poorly studied aspects of radiation effects on blood: potential combination effects of storage and high-dose radiation. The acquired information will contribute to risk assessment of banked blood following accidental radiation with high-dose exposure. Additionally, in vivo irradiation of tissues may produce deleterious effects on nearby tissue cells (i.e., bystander effect), blood, and blood vessels. Knowing the effects of radiation upon each component (e.g., blood) may facilitate interpretation of in vivo results.

### Objectives and Hypotheses

The experiments were designed to examine chemical and cellular effects of cold-storage and irradiation on WB. Multiple biochemical and hematological indices were obtained, while several coagulation and cellular profiles, including differential white cell count, were investigated. We tested the hypothesis that the combined effect of irradiation on stored blood would lead to worse levels of measured parameters compared to nonirradiated samples. To test this hypothesis, we used a higher dose (75 Gy) and higher dose rate (6.7 Gy/min) than most previous studies, which could be reflected in poorer levels of measured indices.

## Materials and Methods

Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AAALAC.

### Animals

Sixteen male Sprague–Dawley rats (body mass: 631 ± 18 g), supplied by a single-source breeder (Charles River, Wilmington, MA, USA), were used as blood donors. These animals were housed in an environment with controlled temperature (20–26°C), humidity (30-70%), and illumination (12 hours light/12 hours dark cycle). Standard diet (Lab Diet 2001, PMI Nutritional International, LLC, Brentwood, MO, USA) and water ad libitum were provided.

### Blood Collection

Blood was carefully removed from a cannulated artery of anesthetized animals (isoflurane 2%, 100% $O_2$) that were breathing spontaneously. Adequacy of anesthesia was assessed by monitoring cardiorespiratory responses to external noxious stimuli. Collected whole blood was anticoagulated with citrate-phosphate-dextrose (CPD, 9:1 ratio) and aliquoted into individually identified 10-mL storage blood minibags (Safe Sens, Blood Cell Storage, Inc., Seattle, WA, USA). Blood bags were randomly assigned to various groups, depending on the protocol (see below). At least 7 bags per group were studied. Each donor animal provided ≈19 mL of blood to the study before low blood pressure or other issues prevented further blood withdrawal. At that point, every anesthetized animal received a lethal dose of euthanasia solution (Fatal-Plus; Vortech Pharmaceuticals, Ltd., Dearborn, MI, USA).

### Experimental Design and Protocols

After collection, blood aliquots were assigned to either 1 of 2 protocols (Figure 1).

In the irradiated fresh blood protocol, aliquots were irradiated on the same day of collection and then stored in a dedicated refrigerator at 4°C without agitation[9,10] for further measurements.

In the irradiated stored blood protocol, aliquots were immediately stored for 3 days at 4°C before irradiation. For both protocols, measurements of cellular and biochemical parameters on irradiated stored blood occurred after either 1 or 3 days after irradiation. A blood sample was also collected from each animal on the day of surgery, before assignment to either protocol, for baseline measurements.

### Radiation Instrument and Irradiations

One self-contained X-ray irradiation system (MultiRad 350, Precision X-Ray, North Branford, CT, USA) was used for all irradiations (Figure 2). Before each irradiation, an automated dose check was performed to assure that the desired radiation

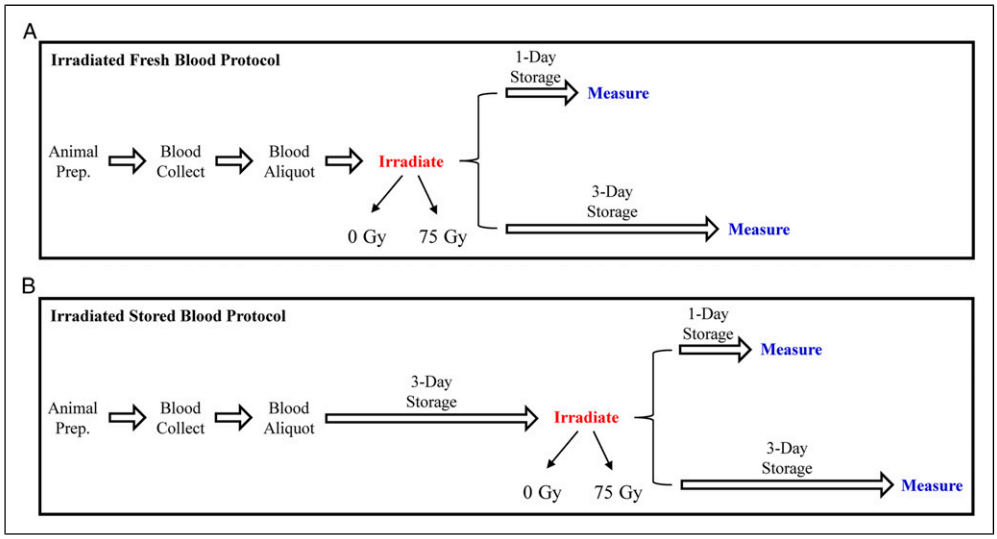


**Figure 1.** Protocols used in the experiments. In all cases, whole blood was collected from anesthetized rats, aliquoted, and stored in blood bags. (A) Some bags were immediately exposed to either 75 Gy radiation or 0 Gy (controls). Measurements of cellular and biochemical parameters were performed after either 1 or 3 days of cold (4°C) storage. (B) After collection, some blood bags were cold-stored for 3 days and then irradiated. Post-irradiation measurements occurred after either 1 or 3 days of cold storage.

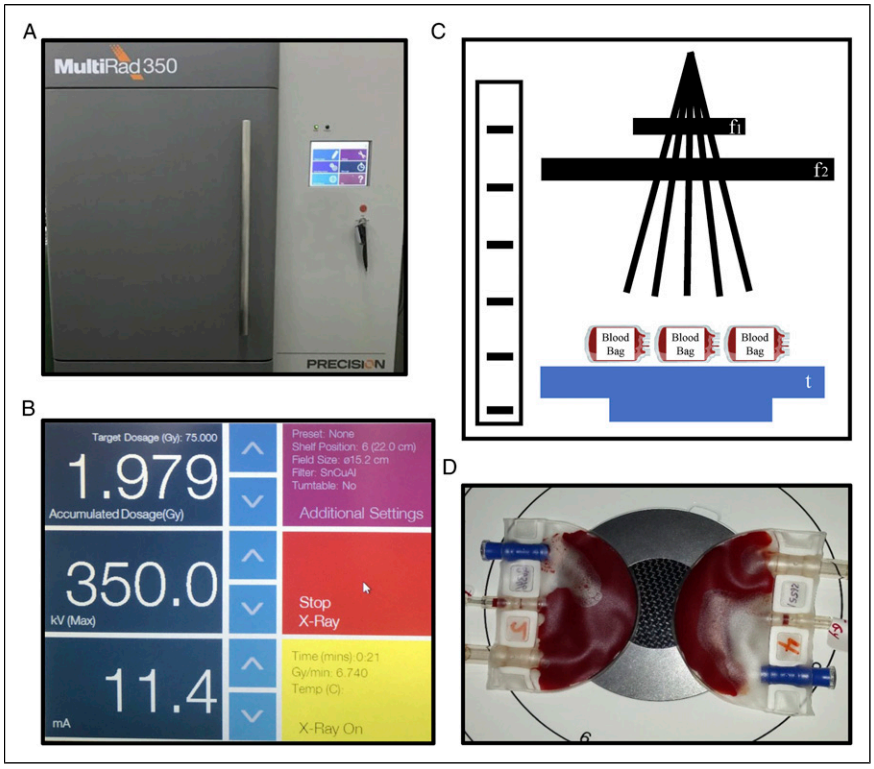


**Figure 2.** Setup used in the experiments. (A) An X-ray irradiation system (MultiRad 350, Precision X-Ray, North Branford, CT, USA) was used with settings (B) of 75 Gy (dose), 6.7 Gy/min (rate), and ≈15 cm (field size). Diagram (C) illustrates the system that included an internal, permanent filter (f1) and an additional tin–copper–aluminum (SnCuAl) filter (f2), placed in the X-ray path, and 1 turntable (t), where 2–4 blood bags were placed. Picture (D) shows an example of a typical experiment with 2 blood bags placed around the dosimeter, on the turntable, inside the cabinet of the irradiation system.

dose was accurately delivered by the equipment. The ion chamber (dosimeter) was positioned at the turntable center during all radiation tests. 2 to 4 blood bags at a time were positioned around the dosimeter and exposed to either 75 Gy at a dose rate of 6.7 Gy/min, or 0 Gy for the same amount of time.

### Measurements

Over 30 parameters were followed to investigate biochemical, hematological, and cellular changes. Heparinized glass microcapillary tubes were used for hematocrit (HCT) measurement. Total hemoglobin concentration (Hb), pH, $K^+$, $Na^+$, $Cl^-$, glucose, bicarbonate ($HCO_3^-$), and lactate levels were measured using a benchtop blood analyzer (ABL 827, Radiometer, Copenhagen, Denmark). Complete blood count and differential white blood cell (WBC) count were determined using a comprehensive hematology system/cell counter (Advia 120, Siemens, Erlangen, Germany).

Platelet aggregation was measured by light transmittance with an aggregometer (Chrono-Log Model 700, Chrono-Log Corp., Havertown, PA, USA), using platelets from platelet-rich plasma and adenosine diphosphate as agonist.[9] Hemostasis was also assessed ex vivo using rotational thromboelastometry (ROTEM, TEM Innovations GmbH, Germany) to measure Extem and Fibtem clotting time (CT), maximum clot firmness (MCF), clot formation time (CFT), and alpha angle.[11] Other parameters in the hemostasis profile that were also measured included fibrinogen, prothrombin time (PT), and activated partial thromboplastin time (aPTT) (Start 4, Diagnostica Stago, Parsippany, NJ, USA).

### Data Presentation and Statistics

Deviation from Gaussian distribution was tested (Shapiro–Wilk test), and nonparametric tests were found adequate for most cases. Values are reported as median [interquartile range, IQR, 25th–75th percentile] or mean ± standard deviation (SD), accordingly. The coefficient of variation (CV), the ratio of SD to the mean, was used as a measure of variability, expressed as a percentage. Differences among more than 2 groups were analyzed using one-way analysis of variance followed by the Holm–Sidak test. When the normality test failed, differences between more than 2 groups were tested using the Kruskal–Wallis test followed by nonparametric tests (Tukey or Dunn method). Power analysis showed that at the standard deviations obtained, a power of .8 was typically reached (alpha = .05) using the group sizes tested. We used commercial software (SigmaPlot 14.0, Systat Software, San Jose, CA; Excel, Microsoft Corp, Redmond, WA). *P* values correspond to two-tailed tests set at .05 significance.

## Results

Baseline measurements showed that the whole blood used for these experiments was within the normal range for all studied parameters, which included a wide spectrum of cellular, biochemical, hematological, and hemostasis indices. The variability of baseline measurements for various parameters, estimated by the CV, was usually below 10%. This indicates that the measurements were performed in a relatively homogenous group of samples and animals.

### Biochemical Changes

As storage time advanced, all parameters changed from their respective baseline values (Figure 3). $Na^+$, glucose, pH, and $HCO_3^-$ progressively decreased with more cold storage time, while $Cl^-$ and lactate levels increased as a function of storage time. Potassium levels followed a similar pattern as $Cl^-$ and lactate (i.e., $K^+$ rapidly increased with storage time). However, multiple times $K^+$ values for stored samples were above measurable levels, and it was not possible to accurately compute valid statistics. The effect of radiation was statistically significant for $Na^+$ in fresh blood samples that were subsequently irradiated (Figure 3A).

### Cellular Changes

White blood cell counts were always reduced following each irradiation, regardless of storage time, although reductions did not reach statistical significance (Table 1). The differential WBC count indicated that most cell types showed reduced number after irradiation, and statistical significance could be demonstrated for monocytes in blood bags stored for 3 days after irradiation (Figure 4). All cell types showed changes after storage, and statistically significant changes could be demonstrated for basophils (Figure 4E). Among 28 pairwise comparisons between 0 and 75 Gy groups for 7 hematological parameters, values were reduced after radiation in most cases (22/28, 79%), but changes were not statistically significant (Table 1). Platelet-related parameters (PLT and MPV) showed increased values after radiation in 4 groups (50%), and RBC were higher for 75 Gy treatments in the 3-day prior/3-day post group.

### Hematology and Hemostasis

Since reductions in RBC number were relatively small, corresponding changes in hematological indices such as HCT and Hb were also unimportant (Table 1). Although alterations in platelet number were relatively small, platelet function was significantly modified in stored samples. Aggregometry tests showed that storage time had an inhibitory effect on aggregation as expressed by progressively lower values of amplitude, area under the curve, and slope (Table 2) except for 3-day prior/75 Gy treatment, where the value is lower in the 1-day post-group than in the 3-day post-group. These values for aggregation were numerically higher for 3-day stored samples after radiation, but statistical significance was not reached. Fibtem and Extem ROTEM parameters such as CT, CFT, MCF, and alpha angle were not significantly altered by either storage time or radiation (Table 2). Likewise, other significant hemostasis indices such as fibrinogen, PT, and aPTT were not significantly changed.

## Discussion

Despite its critical importance, combined effects of storage and radiation on blood have been poorly documented. Our knowledge on storage and irradiation derives mostly from

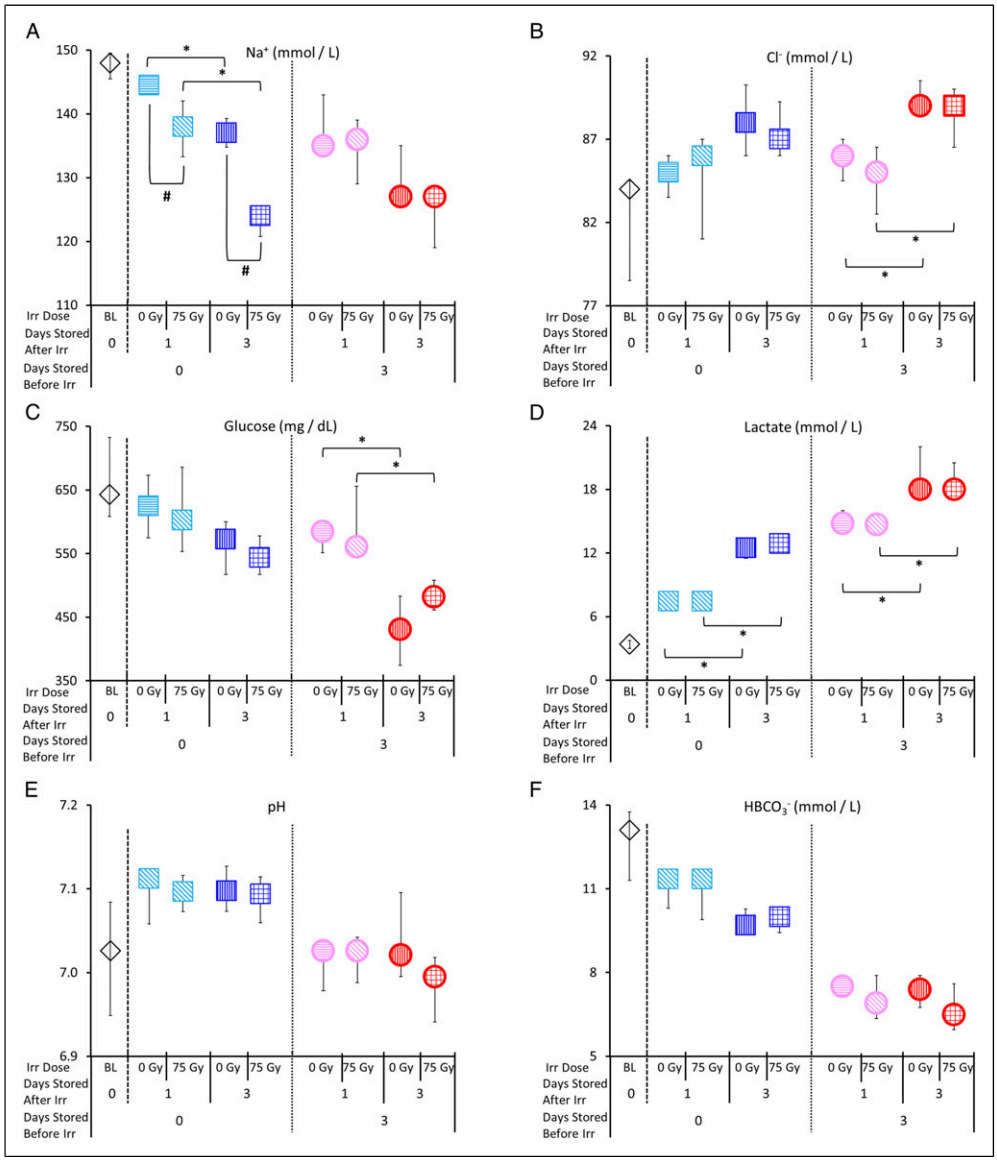


**Figure 3.** Blood biochemical changes after cold storage and irradiation. As a trend, Na+, glucose, pH, and bicarbonate (HCO3-) progressively decreased with cold storage time, while Cl- and lactate levels increased as storage time advanced. For Na+, some pairwise comparisons of irradiated samples against controls were statistically significant (A). BL, baseline. Data are median with interquartile ranges. * Significantly different from blood bags stored for less time (3 days vs 1 day). # Significantly different from blood bags stored for similar periods of time but not irradiated (75 Gy vs 0 Gy).

studies on their isolated effects upon blood.[12,13] This study provides new data on combined effects of high-dose irradiation and storage upon blood. Blood was irradiated and then cold-stored up to 3 days or cold-stored for up to 3 days and then irradiated. To our knowledge, this is the most complete study on combined effects of irradiation and storage on blood components to date.

However, irradiation of collected blood has been used to prevent transfusion-associated graft-versus-host disease.[14] In these cases, blood has been irradiated frequently using gamma rays and lower doses than those used in our study (25-50 Gy). Only limited scope studies have been performed combining storage and radiation, for example, using human neutrophils and low-dose radiation.[15] The overall conclusion from these studies was that irradiation of cellular components was safe for general use. The number and range of parameters measured in these initial studies were relatively limited. More recent and specific studies on X-ray irradiated (35 Gy) RBCs have found that storage lesion was accelerated by irradiation.[16] We used a higher radiation dose and dose rate than all previous studies, and the range of parameters evaluated was relatively wide. Special attention was given to hemostasis since whole blood is the gold standard for resuscitation after traumatic hemorrhage[2,3] and because coagulopathy is a major issue.[17]

**Table 1.** Hematological Parameters After Whole Blood Cold Storage and Irradiation.

| Storage Time prior Irradiation | 0 Days | | | | | 3 Days | | | |
|---|---|---|---|---|---|---|---|---|---|
| Storage Time after Irradiation | — | 1 Day | | 3 Days | | 1 Day | | 3 Days | |
| Irradiation Dose | — | 0 Gy | 75 Gy | 0 Gy | 75 Gy | 0 Gy | 75 Gy | 0 Gy | 75 Gy |
| Parameter ↓ | Baseline | | | | | | | | |
| WBC ($10^3$ cells/μL) | 3.8 ± 1.6 | 3.8 ± 1.0 | 3.5 ± 0.9 | 4.8 ± 1.3 | 4.4 ± 0.9 | 3.3 ± 1.7 | 3.2 ± 1.2 | 5.6 ± 1.6 | 5.0 ± 1.7 |
| RBC ($10^6$ cells/μL) | 6.4 ± 0.6 | 6.8 ± 1.5 | 6.4 ± 1.1 | 7.8 ± 1.6 | 7.8 ± 1.7 | 6.5 ± 0.5 | 6.2 ± 1.0 | 7.0 ± 0.8 | 7.8 ± 2.4 |
| Hb (g/dL) | 10.9 ± 0.8 | 11.7 ± 2.1 | 10.9 ± 1.4 | 13.4 ± 2.8 | 13.3 ± 2.6 | 11.7 ± 1.1 | 10.8 ± 1.4 | 12.6 ± 1.3 | 12.2 ± 1.2 |
| HCT (%) | 33.2 ± 2.5 | 34.6 ± 7.1 | 31.0 ± 4.4 | 39.0 ± 9.3 | 37.1 ± 8.3 | 32.2 ± 3.1 | 29.4 ± 4.6 | 34.2 ± 4.0 | 32.9 ± 3.9 |
| MCV ($\mu m^3$) | 52.1 ± 2.3 | 51.0 ± 2.3 | 49.1 ± 2.3 | 49.7 ± 2.2 | 47.5 ± 2.0 | 48.7 ± 1.9 | 47.6 ± 1.8 | 48.9 ± 1.7 | 47.9 ± 1.8 |
| PLT ($10^3$ cells/μL) | 881 ± 117 | 748 ± 149 | 795 ± 149 | 843 ± 96 | 819 ± 121 | 868 ± 368 | 902 ± 172 | 769 ± 297 | 766 ± 173 |
| MPV (fL) | 9.6 ± 0.6 | 6.8 ± 0.5 | 7.1 ± 0.4 | 7.3 ± 1.7 | 7.7 ± 1.9 | 11.3 ± 7.5 | 8.5 ± 3.1 | 13.1 ± 4.1 | 11.7 ± 4.1 |

Values are mean ± SD, n = 8-15 per group.
WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin concentration; HCT, hematocrit; MCV, mean corpuscular volume; PLT, platelets; MPV, mean platelet volume.

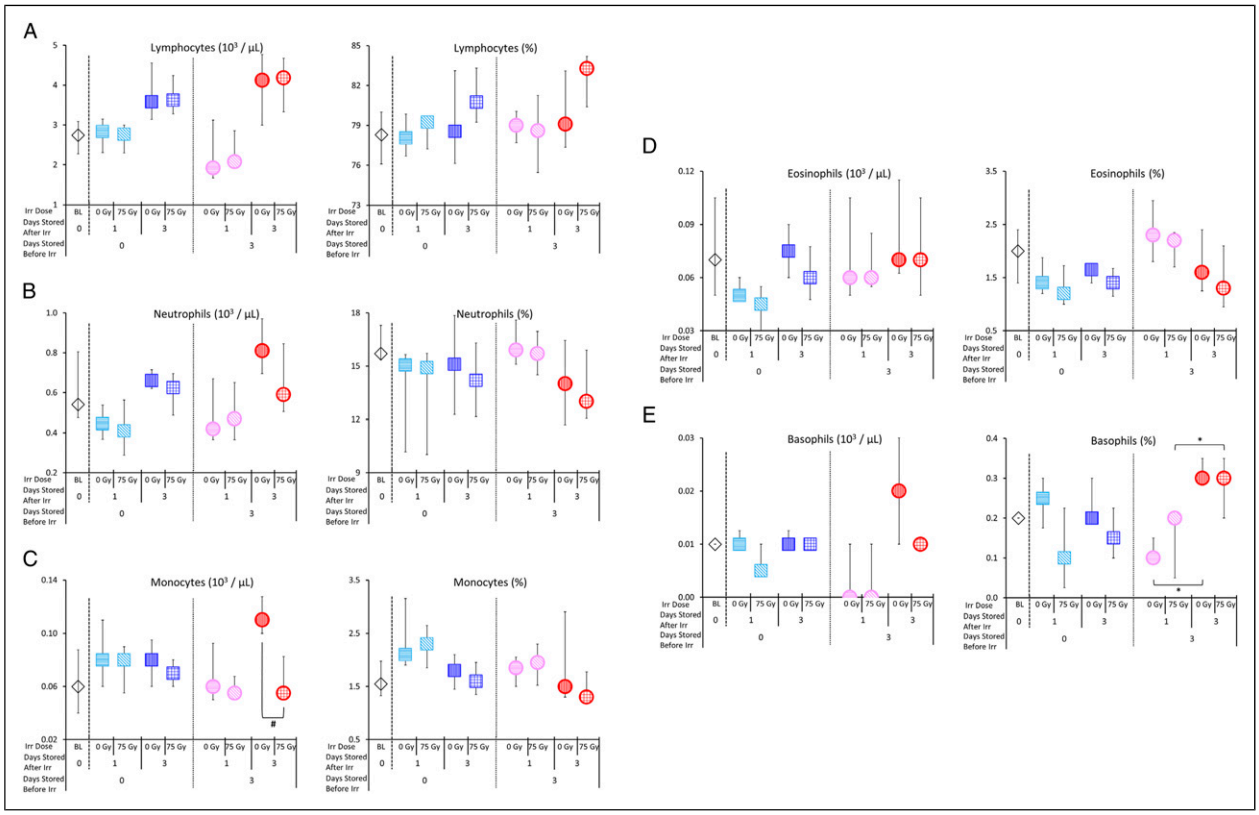


**Figure 4.** Differential white blood cell count after cold storage and irradiation. As a trend, all cell types showed changes after storage, although statistically significant changes could be demonstrated only for basophils (E). Most cell types showed reduced number after irradiation, but statistical significance could be demonstrated only for monocytes in blood stored for 3 days after irradiation (C). BL, baseline. Data are median with interquartile ranges. * Significantly different from blood bags stored for less amount of time (3 days vs 1 day). # Significantly different from blood bags stored for similar periods of time but not irradiated (75 Gy vs 0 Gy).

While some effects were noted in our study, most were associated with storage time alone, and only a small number were significantly altered by irradiation. Therefore, the irradiation level of 75 Gy did not seem to significantly impact the changes of most parameters studied in whole blood stored up to 3 days.

**Table 2.** Hemostasis and Platelet Aggregation Parameters After Whole Blood Cold Storage and Irradiation.

| Storage Time prior Irradiation | 0 Days | | | | | 3 Days | | | |
|---|---|---|---|---|---|---|---|---|---|
| Storage Time after Irradiation | — | 1 Day | | 3 Days | | 1 Day | | 3 Days | |
| Irradiation Dose | — | 0 Gy | 75 Gy | 0 Gy | 75 Gy | 0 Gy | 75 Gy | 0 Gy | 75 Gy |
| Parameter ↓ | Baseline | | | | | | | | |
| Fibrinogen (mg/dL) | 157.7 ± 14.6 | 158.4 ± 17.4 | 157.0 ± 16.5 | 166.4 ± 15.6 | 170.4 ± 20.9 | 150.3 ± 14.1 | 148.3± 21.8 | 174.4 ± 29.4 | 168.3 ± 15.6 |
| PT (s) | 21.4 ± 1.7 | 18.9 ± 1.3 | 19.4 ± 1.7 | 18.4 ± 0.9 | 18.7± 0.9 | 19.1± 1.6 | 19.5 ± 1.8 | 17.3 ± 1.0 | 18.4 ± 1.5 |
| aPTT (s) | 25.4 ± 13.1 | 40.1 ± 19.3 | 41.2 ± 20.1 | 49.7 ± 29.7 | 49.0 ± 26.3 | 48.1 ± 8.1 | 49.3 ± 9.0 | 43.6 ± 9.9 | 48.4 ± 5.7 |
| ROTEM | | | | | | | | | |
| Extem CT (s) | ND | 36.6 ± 12.5 | 40.9 ± 8.2 | 52.6 ± 13.9 | 47.8 ± 14.1 | 39.6 ± 2.6 | 36.1 ± 4.5 | 43.2 ± 7.3 | 33.9 ± 7.5 |
| Fibtem CT (s) | ND | 41.9 ± 8.0 | 38.7 ± 6.5 | 42.8 ± 11.7 | 47.8 ± 21.7 | 31.2 ± 2.5 | 34.4 ± 2.2 | 39.9 ± 4.1 | 37.3 ± 6.7 |
| Extem CFT (s) | ND | 58.5 ± 17.1 | 61.3 ± 14.7 | 80.2 ± 22.8 | 74.4 ± 25.1 | 67.6 ± 12.5 | 67.2 ± 12.3 | 84.1 ± 19.4 | 62.4 ± 12.4 |
| Extem MCF (mm) | ND | 53.9 ± 10.4 | 57.4 ± 4.1 | 58.1 ± 3.8 | 58.8 ± 3.3 | 56.5 ± 2.7 | 55.5 ± 4.3 | 55.9 ± 5.7 | 56.1 ± 6.7 |
| Fibtem MCF (mm) | ND | 16.1 ± 3.3 | 15.8 ± 2.4 | 18.8 ± 2.6 | 17.9 ± 3.3 | 18.0 ± 4.8 | 15.1 ± 2.0 | 16.6 ± 3.0 | 14.6 ± 2.0 |
| Extem alpha angle (degrees) | ND | 78.4 ± 3.7 | 77.6 ± 2.8 | 74.3 ± 3.7 | 75.3 ± 4.6 | 76.6 ± 2.0 | 77.1 ± 2.4 | 74.1 ± 3.0 | 79.1 ± 5.8 |
| Fibtem alpha angle (degrees) | ND | 69.2 ± 6.1 | 70.9 ± 3.7 | 73.3 ± 6.8 | 74.5 ± 3.0 | 77.3 ± 5.2 | 73.4 ± 3.1 | 68.0 ± 7.0 | 70.4 ± 8.3 |
| Aggregation | | | | | | | | | |
| Amplitude (%) | ND | 61.0 [50.3-71.3] | 48.0 [43.5-65.3] | 42.0 [24.3-58.5] | 44.0 [40.0-50.5] | 50.0 [43-60] | 44.0 [32.0-54.0] | 17.0 [9.0-38.0] | 31.5 [26.3-45.5] |
| Slope (AU/min) | ND | 98.5 [77.5-138.3] | 90.5 [57.3-102.5] | 69.0 [44.0-117.5] | 79.0 [61.5-123] | 99.0 [96-131] | 74.0 [68.0-77.0] | 18.0 [15.0-77.0] | 79.5 [57.0-86.8] |
| AUC | ND | 480 [140-701] | 455 [365-637] | 399 [231-623] | 406 [314-439] | 557 [484-650] | 371 [280-520] | 122 [78-415] | 308 [220-452] |
| Lag time (s) | ND | 10.0 [8.3-13.5] | 10.5 [8.5-23.0] | 16.0 [10.0-35.8] | 12.0 [10.0-15.0] | 10.0 [8.0-18.0] | 12.0 [10.0-25.0] | 18.0 [11.3-55.5] | 18.0 [6.0-46.0] |

Values are mean ± SD or median [IQR], n = 8–15 per group.

ND, not determined; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; AUC, area under the curve; PT, prothrombin time; aPTT, activated partial thromboplastin time; ROTEM, rotational thromboelastometry. Significantly different from 0 Gy dose, 1 day storage after irradiation, 3 days prior irradiation.

When irradiation studies are performed in vivo,[18] interpretation of changes in peripheral blood cells is difficult due to multiple and complex actions occurring to other structures interacting with blood cells. Although limited in scope, ex vivo studies using irradiated whole blood, as in the current work, allow separating the direct effects of radiation solely on the blood compartment.

Although the anticoagulant used lacked adenosine, which would support cell metabolism and help counteract RBC storage lesion, the data showed little RBC damage. As noted below, this could be partially due to the limited duration of the storage. The observed RBC resistance to high dose ionizing radiation could possibly be explained by the presence of high oxygen concentrations in blood bags, maintaining Hb iron in a reduced state (i.e., methemoglobin).[19,20]

In our study, alterations associated with storage were observed in several parameters. It has been suggested that species-specific differences in the structure and metabolism could be responsible for storage lesion that is 4 times faster in rats than in humans.[21] Therefore, a 3-day storage would be approximately equivalent to 12 days. Only in some cases, we measured incremental changes that were apparently induced by irradiation.

Our total platelet count, white cell count, and leukocyte differential results were in agreement with previously published measurements for rats.[9,22,23] We observed only small alterations in WBC and PLT although PLT counts have been reported to decrease by 1–2% per day in cold-stored WB.[4] Most cell types showed reduced number after irradiation, and statistical significance was demonstrated for monocytes stored for 3 days after irradiation. Interestingly, higher sensitivity to radiation specific for monocytes has been previously reported following in vitro irradiation.[24]

Albumin may have contributed to the overall resistance to high-dose ionizing radiation observed in our study. Generally, albumin constitutes the major plasma protein target of oxidant stress. Unlike red blood cell concentrates, the plasma present in whole blood bags is less diluted (without filtration and addition of additives). In our experiments, the presence of a relatively normal albumin concentration in WB can decrease oxidative stress caused by high-dose X-ray radiation, since albumin represents the major and predominant antioxidant in plasma.[25]

## *Limitations and Applications*

This study has several limitations that we must acknowledge, including the limited number of irradiation levels, and the fact that we did not use leukoreduced samples. However, the leukoreduction process removes most white cells and platelets.[26,27] By using nonleukoreduced blood, unique data were provided on storage and radiation effects on platelets, WBC, and differential WBC count. While platelet function was evaluated, functional assessments of white cells were not performed. We used CPD as anticoagulant, while CPDA-1 is commonly used for storage, particularly for its beneficial effects on RBCs.[28] However, we noted only nonsignificant changes in RBC, possibly due to the limited storage time in our study.

## *Conclusions*

Assessment of a wide range of cellular and biochemical parameters showed that alterations observed are primarily associated with storage. Only in a few instances, these changes are further altered by radiation, likely because mechanisms of cellular injury caused by high-dose X-ray radiation are mostly indirect. Data suggest that cold-stored blood can sustain up to 75 Gy radiation without major changes in critical parameters and therefore remain suitable for in vivo use. Evaluating fresh and cold-stored blood parameters after radiation ex vivo aids in interpreting radiation effects on blood/blood vessel systems in vivo and assessment of stored blood adequacy after radiation exposure.

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## Author Contributions

I.P.T.F. designed the study. D.B., C.W., and K.H. performed the research. D.B., C.W., K.H., L.N.T., and I.P.T.F. analyzed the data and interpreted the results. I.P.T.F. drafted the paper and prepared all figures and tables. All authors critically reviewed and approved the manuscript.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## References

1. Nenot JC. Radiation accidents: Lessons learnt for future radiological protection. *Int J Radiat Biol*. 1998;73(4):435-442.
2. Cap AP, Beckett A, Benov A, et al. Whole blood transfusion. *Mil Med*. 2018;183(suppl 2):44-51.
3. Hazelton JP, Cannon JW, Zatorski C, et al. Cold-stored whole blood: A better method of trauma resuscitation? *J Trauma Acute Care Surg*. 2019;87(5):1035-1041.
4. van der Meer PF, Klei TR, de Korte D. Quality of platelets in stored whole blood. *Transfus Med Rev*. 2020;34(4):234-241.

5. Wang Z, Lv MY, Huang YX. Effects of low-dose X-ray on cell growth, membrane permeability, DNA damage and gene transfer efficiency. *Dose Response*. 2020;18(4):1–11.
6. Belkacemi Y, Bouchet S, Frick J, et al. Monitoring of residual hematopoiesis after total body irradiation in humans as a model for accidental x-ray exposure: Dose-effect and failure of ex vivo expansion of residual stem cells in view of autografting. *Int J Radiat Oncol Biol Phys*. 2003;57(2):500-507.
7. Saikkonen A, Niemela J, Sipila P, Keyrilainen J. Commissioning of the MultiRad 350 cell and small animal x-ray irradiation system. *Phys Med*. 2019;59:107-111.
8. Andersen AHF, Nielsen SSF, Olesen R, et al. Comparable human reconstitution following Cesium-137 versus X-ray irradiation preconditioning in immunodeficient NOG mice. *PLoS One*. 2020;15(10):e0241375.
9. Torres Filho IP, Torres LN, Valdez C, Salgado C, Cap AP, Dubick MA. Refrigerated platelets stored in whole blood up to 5 days adhere to thrombi formed during hemorrhagic hypotension in rats. *J Thromb Haemostasis*. 2017;15(1):163-175.
10. Yazer MH, Glackin EM, Triulzi DJ, Alarcon LH, Murdock A, Sperry J. The effect of stationary versus rocked storage of whole blood on red blood cell damage and platelet function. *Transfusion*. 2016;56(3):596-604.
11. Torres Filho IP, Barraza D, Williams C, Hildreth K, Dubick MA. Automated noninvasive evaluation of blood flow and oxygenation in rats integrated with systemic physiological monitoring. *J Trauma Acute Care Surg*. 2019;87(1S suppl 1):S110-S118.
12. Fischer F, Aulmann M, Maier-Borst W, Lorenz WJ. Blood cell damage after in vitro irradiation of fresh whole blood with 630 nm laser light. *Blood Cells Mol Dis*. 1998;24(3):385-395.
13. Logan ID, Barnett YA. A comparison between the application of the comet assay and an immunochemical DNA damage assay on the level of DNA damage within X-ray irradiated human whole blood. *Biochem Soc Trans*. 1998;26(1):S86.
14. Przepiorka D, LeParc GF, Stovall MA, Werch J, Lichtiger B. Use of irradiated blood components: Practice parameter. *Am J Clin Pathol*. 1996;106(1):6-11.
15. Buescher ES, Gallin JI. Effects of storage and radiation on human neutrophil function in vitro. *Inflammation*. 1987;11(4):401-416.
16. Suzuki Y, Tateishi N, Cicha I, et al. Decreased deformability of the X-ray-irradiated red blood cells stored in mannitol-adenine-phosphate medium. *Clin Hemorheol Microcirc*. 2000;22(2):131-141.
17. Darlington DN, Craig T, Gonzales MD, Schwacha MG, Cap AP, Dubick MA. Acute coagulopathy of trauma in the rat. *Shock*. 2013;39(5):440-446.
18. Ghandhi SA, Turner HC, Shuryak I, et al. Whole thorax irradiation of non-human primates induces persistent nuclear damage and gene expression changes in peripheral blood cells. *PLoS One*. 2018;13(1):e0191402.
19. Antonelou MH, Kriebardis AG, Stamoulis KE, Economou-Petersen E, Margaritis LH, Papassideri IS. Red blood cell aging markers during storage in citrate-phosphate-dextrose-saline-adenine-glucose-mannitol. *Transfusion*. 2010;50(2):376-389.
20. Yoshida T, Prudent M, D'Alessandro A. Red blood cell storage lesion: Causes and potential clinical consequences. *Blood Transfus*. 2019;17(1):27-52.
21. d'Almeida MS, Jagger J, Duggan M, White M, Ellis C, Chin-Yee IH. A comparison of biochemical and functional alterations of rat and human erythrocytes stored in CPDA-1 for 29 days: Implications for animal models of transfusion. *Transfus Med*. 2000;10(4):291-303.
22. Faas MM, Moes H, van der Schaaf G, de Leij LF, Heineman MJ. Total white blood cell counts and LPS-induced TNF alpha production by monocytes of pregnant, pseudopregnant and cyclic rats. *J Reprod Immunol*. 2003;59(1):39-52.
23. Suzuki S, Eguchi N. Leukocyte differential analysis in multiple laboratory species by a laser multi-angle polarized light scattering separation method. *Exp Anim*. 1999;48(2):107-114.
24. Buescher ES, Gallin JI. Radiation effects on cultured human monocytes and on monocyte-derived macrophages. *Blood*. 1984;63(6):1402-1407.
25. Roche M, Rondeau P, Singh NR, Tarnus E, Bourdon E. The antioxidant properties of serum albumin. *FEBS Lett*. 2008;582(13):1783-1787.
26. Serrano K, Levin E, Culibrk B, et al. Performance characteristics of a novel blood bag in-line closure device and subsequent product quality assessment. *Transfusion*. 2010;50(10):2240-2248.
27. Vinholte BP, Sousa RDS, Assis FFV, et al. The effects of pre-storage leukoreduction on the conservation of bovine whole blood in plastic bags. *Biology (Basel)*. 2020;9(12):444.
28. Meledeo MA, Peltier GC, McIntosh CS, Bynum JA, Cap AP. Optimizing whole blood storage: Hemostatic function of 35-day stored product in CPD, CP2D, and CPDA-1 anticoagulants. *Transfusion*. 2019;59(suppl 2):1549-1559.