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POSTTRANSFUSION SURVIVAL AND DISTRIBUTION OF 51-CR LABELED
FRESH AND LIQUID PRESERVED SYNGENEIC MOUSE RED BLOOD CELLS

BY

C.R. VALERI, T. ICHIKURA, L.E. PIVACEK, S. PRUSTY, AND
J. DITTMER

NAVAL BLOOD RESEARCH LABORATORY
BOSTON UNIVERSITY SCHOOL OF MEDICINE
615 ALBANY STREET
BOSTON, MA 02118

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established in 24 mice: $RCV, ml = 0.017 BW + 0.29$. The blood volume of each organ was measured and related to organ weight.

Heparinized RBC were labeled with ^{51}Cr after storage at 4 C for 11 to 24 days and infused. Radioactivity was measured in the injectate and in post-infusion blood and organ samples. RBC survival was calculated from the injectate and the blood sample radioactivity, and from the theoretical red cell volume based on body weight. The 24-hour posttransfusion survival was about 75% for red blood cells stored at 4 C for 11 days and about 30% for red blood cells stored at 4 C for 24 days. The organ radioactivity was corrected for intravascular radioactivity using the theoretical organ blood volume based on organ weight. The extravascular radioactivity in each organ was calculated as % of total radioactivity infused and % of nonviable RBC radioactivity infused. The organ radioactivity increased with the percentage of nonviable RBC infused and the length of storage at 4 C. In 28 studies, the principal sites of sequestration were the liver, spleen, and skeleton. Twenty-four hours after transfusion, the percentages of nonviable RBC in the liver, spleen, and skeleton were 31 ± 7 , 25 ± 6 , and 30 ± 5 , respectively. The sequestration per gram of tissue was greater in the spleen and bone marrow than in the liver. Approximately 100% of the infused radioactivity was recovered in the blood, organs, and urine.

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LIQUID PRESERVED SYNGENEIC MOUSE RED BLOOD CELLS

Valeri CR, Ichikura T, Pivacek LE, Prusty S, Dittmer J

ABSTRACT

Recent studies suggest that the transfusion of nonviable red blood cells (RBC) inhibits the reticuloendothelial system. The study reported here examines the *in vivo* distribution of nonviable RBC transfused in syngeneic mice.

Within 1 hour after the transfusion of fresh ^{51}Cr -labeled RBC, blood samples, and liver, spleen, skeleton, lungs, heart and kidneys were collected. The blood and organ samples and injectate were measured for radioactivity. The red cell volume as a function of grams of body weight (BW) was established in 24 mice: $\text{RCV, ml} = 0.017 \text{ BW} + 0.29$. The blood volume of each organ was measured and related to organ weight.

Heparinized RBC were labeled with ^{51}Cr after storage at $+4^\circ\text{C}$ for 11 to 24 days and infused. Radioactivity was measured in the injectate and in post-infusion blood and organ samples. RBC survival was calculated from the injectate and the blood sample radioactivity, and from the theoretical red cell volume based on body weight. The 24-hour posttransfusion survival was about 75% for red blood cells stored at $+4^\circ\text{C}$ for 11 days and about 30% for red blood cells stored at $+4^\circ\text{C}$ for 24 days. The organ radioactivity was corrected for intravascular radioactivity using the theoretical organ blood volume based on organ weight. The extravascular radioactivity in each organ was calculated as % of total radioactivity infused and % of nonviable RBC radioactivity infused. The organ radioactivity increased with the

percentage of nonviable RBC infused and the length of storage at +4C. In 28 studies, the principal sites of sequestration were the liver, spleen, and skeleton. Twenty-four hours after transfusion, the percentages of nonviable RBC in the liver, spleen, and skeleton were 31 ± 7 , 25 ± 6 , and 30 ± 5 , respectively. The sequestration per gram of tissue was greater in the spleen and bone marrow than in the liver. Approximately 100% of the infused radioactivity was recovered in the blood, organs, and urine.

INTRODUCTION

Homologous blood transfusions may be necessary in severe trauma and major surgical operations, but they may have unfavorable effects. In addition to the transmission of infectious diseases, homologous blood may also produce hemolytic reactions, allergic reactions, and immune suppression. Although homologous blood transfusions may prolong organ transplants (1-7), there have been reports of the adverse effects of blood transfusions on the prognosis after curative operation for colorectal cancer, breast cancer, lung cancer, and renal cancer (8-21). Retrospective analyses have shown a correlation between perioperative blood transfusions and postoperative infectious complications (22,23). These observations suggest that immune suppression may occur after blood transfusions, although the exact mechanisms of this is not known. It has been postulated that one immunosuppressive effect of blood transfusions is the nonspecific inhibition of the reticuloendothelial (RE) system which may be induced by the phagocytosis of nonviable red blood cells.

During storage in the liquid state at +4°C, red blood cells are irreversibly damaged and are removed from the circulation during the first 24 hours after transfusion, although the exact sites of removal have never been studied.

Studies in experimental animals have shown that silica and carrageenan, which injure and destroy macrophages, inhibit the function of the RE system and improve graft survival (24,25,26). It has further been reported that silica, carrageenan, and gold salts enhance lung metastases in animal models (27,28,29). If stored red blood cells that are nonviable are removed by macrophages in the RE system, they might adversely affect the immune function.

It has been reported that red blood cells aging in the circulation, as well as heat-damaged and antibody-coated red blood cells, are removed by mononuclear phagocytes in the RE system following infusion (30-37). It has yet to be determined which part of the RE system is the most active in the removal of the red blood cells that are damaged during liquid preservation. In this study, syngeneic mouse red blood cells were stored at 4C in heparin or in the acid citrate dextrose (ACD) anticoagulant, and 24-hour posttransfusion survival and lifespan values were measured and the sites of removal of the non viable red blood cells were assessed.

MATERIALS AND METHODS

Animals

Male B6C3HF1 (C57BL/6) and female (X C3H/He male) mice up to six months of age were studied. The mice were obtained from Jackson Laboratories, Bar Harbor, ME. The genetic integrity of the mice was established using electrophoretic testing for biochemical markers, serological testing for immunological markers, and skin grafting. Histocompatibility was confirmed at our laboratory using dorsal skin grafts on 15 pairs of mice. A graft survival of 60 days was used to assess compatibility.

Collection and Storage of the Mouse Blood

The mice were weighed and anesthetized by intraperitoneal injections of chloral hydrate (0.5 mg / gram of body weight). Each mouse was exsanguinated by withdrawing a 1 to 2 ml volume of blood into a heparinized syringe. The blood was collected by aortic puncture through a laparotomy incision. Fresh blood samples collected from 6 mice were pooled to obtain an 8 ml volume in a sterile glass tube containing either heparin (143 USP units per 10 ml of blood) or acid-citrate-dextrose (ACD, 1.5 ml per 10 ml of blood). Fresh blood collected from 13 to 20 mice was pooled to obtain a 20 to 30 ml volume and was anticoagulated with heparin or ACD in a sterile glass tube and stored at 4C for as long as 24 days.

⁵¹Cr Labeling of Red Blood Cells

The pool of mouse blood samples was centrifuged in a Sorvall GLC-1 at 2400 RPM (590 X g) for 6-8 minutes and the plasma was removed. The red blood cells were washed twice as follows: the red

cell concentrate was diluted to approximately 3 times its volume with 0.9% sodium chloride, then centrifuged at 2400 RPM (590 X g) for 6-8 minutes and the supernatant removed. The red blood cells were resuspended with 0.9% sodium chloride to a hematocrit of 80% and incubated for 30 minutes at 37°C with 1 to 2 uCi ^{51}Cr disodium chromate per ml (ER Squibb Diagnostics, Princeton, NJ). The ^{51}Cr labeled red blood cell suspension was centrifuged at 2400 RPM (590 X g) for 6-8 minutes and the supernatant removed. The red cells were washed twice with 0.9% sodium chloride and resuspended with 0.9% sodium chloride to a hematocrit of 40%.

Red blood cells stored for longer than 11 days were washed up to 4 times before labeling with ^{51}Cr and 3 times after incubation to remove the supernatant hemoglobin. A 4 ml volume of the labeled red cell suspension was used to count the radioactivity in a gamma counter, and the remainder was used for infusion. The loss of hemoglobin from the red blood cells during 4°C storage and during the ^{51}Cr labeling procedure was measured. The supernatant from the red blood cell washing procedure was obtained before and during ^{51}Cr labeling, and the volume and supernatant hemoglobin concentration were measured. The percent hemolysis was calculated from total milligrams of supernatant hemoglobin and total milligrams of cellular hemoglobin.

Infusion of ^{51}Cr -Labeled Red Blood Cells

The mice were weighed and anesthetized with chloral hydrate, and then injected retro-orbitally with a 0.25 ml volume of the ^{51}Cr labeled red blood cell suspension. Up to 20 mice were infused with the ^{51}Cr -labeled syngeneic red blood cell to obtain post-infusion blood and organ samples for testing.

Blood and Organ Sample Collection

For each blood sample obtained following the infusion of ^{51}Cr -labeled red blood cells, it was necessary to exsanguinate two to four of the anesthetized mice: a 1 to 2 ml volume of blood was collected into a heparinized syringe from each mouse by an aortic puncture. The blood samples were pooled to obtain a 4 ml volume, and the average body weight of the mice was recorded. At each post-infusion period, the spleen, liver, kidneys, heart, lungs, skeleton, gastrointestinal tract and skin were removed from one mouse. The organs were removed as quickly as possible, and the mouse was sacrificed by cervical dislocation. The soft tissue was removed from the skeleton to obtain the skin, muscle, and skeleton samples. All the organ samples were weighed unwashed prior to measurement of their radioactivity.

Radioactivity Measurements

One ml of the ^{51}Cr -radiolabeled injectate, 1 ml of a 1:50 dilution of the injectate, 0.5 ml of the supernatant of the injectate, and 1 ml blood and 0.5 ml plasma from each post-infusion blood sample were pipetted into gamma counting tubes. When organ samples were obtained, each organ or organ pair was weighed and placed into a gamma counting tube. All the blood and organ samples collected from the series of mice infused with a ^{51}Cr -labeled injectate were counted together with the injectate sample in a sodium iodide well-type scintillation counter (Model 1185, TM Analytic Inc, Elk Grove Village, IL).

Red Cell Volume Determinations

In each of 8 studies in which fresh heparin blood was pooled and the red blood cells labeled with ^{51}Cr , 3 mice were infused with 0.25

ml of the ^{51}Cr -labeled fresh red blood cell suspension. Ten (10) minutes following the infusion, blood samples from the three mice were collected and pooled. Measurements were made of the hematocrits of the labeled red blood cell suspensions and the post infusion blood samples. Aliquots were centrifuged at 2400 RPM (590 X g) for 6-8 minutes, and the plasma was obtained. Radioactivity was measured in the injectate samples and in the blood and plasma samples.

Radioactivity is expressed as counts per minute (cpm), and hematocrit (hct) as a decimal in the calculations that follow.

The radioactivity associated with the infused red blood cells was determined from the radioactivity in the injectate and the supernatant of the injectate, and the hematocrit, as follows:

$$(a) \text{ INFUSED RBC RADIOACTIVITY, cpm} = [\text{INJECTATE, cpm/ml} - (\text{SUPNT, cpm/ml} (1-\text{hct}))] \times 0.25 \text{ ml}$$

The radioactivity of the red blood cells in the post infusion sample was calculated using the radioactivity of the blood and the plasma as follows:

$$(b) \text{ POST-INFUSION RBC RADIOACTIVITY, cpm/ml} = \frac{\text{BLOOD, cpm/ml} - (\text{PLASMA, cpm/ml} (1-\text{hct}))}{\text{hct}}$$

The red cell volume, total blood volume, and plasma volume of the mouse were calculated using the infused red cell radioactivity (a), the post infusion red blood cell radioactivity (b), and the hematocrit as follows:

$$(c) \text{ RED BLOOD CELL VOLUME, ml} = \frac{\text{INFUSED RBC, cpm}}{\text{POST INFUSION RBC, cpm/ml}}$$

$$(d) \text{ TOTAL BLOOD VOLUME, ml} = \frac{\text{RED CELL VOLUME, ml}}{\text{hct}}$$

$$(e) \text{ PLASMA VOLUME, ml} =$$

TOTAL BLOOD VOLUME, ml - RED BLOOD CELL VOLUME, ml

Linear regression analysis was used to determine the relationship between red cell volume and body weight, and the relationship between total blood volume and body weight.

The hematocrits used in the calculations were measured on 1 to 2 ml volume samples obtained from the abdominal aorta following the infusion of the ^{51}Cr -labeled red blood cell suspension. The total body hematocrit was measured in blood samples obtained after exsanguination. Hematocrit values were measured in a group of mice on a 1.2 ml volume of blood obtained from the abdominal aorta, a 0.2 ml volume of blood obtained from the abdominal aorta, and a 0.2 ml volume of blood obtained from the inferior vena cava.

In Vivo Survival Measurement of Liquid Preserved Red Cells

In 22 experiments heparinized blood was stored at +4C for 0 to 24 days, and in 2 experiments ACD blood was stored for 0 and 15 days prior to ^{51}Cr labeling. For each study, three to nine mice were each infused with 0.25 ml of the ^{51}Cr red cell suspension. Heparinized blood from 3 mice was pooled for each blood sample: the samples were obtained 10 minutes, sometimes 1 hour, and 24 hours following transfusion. Hematocrit measurements were made on the radiolabeled red blood cell injectates and post-infusion blood samples. The blood samples were centrifuged at 2400 RPM (590 X g) for 6-8 minutes, and the supernatant or plasma was obtained. Radioactivity was measured in the injectate samples and in the post-infusion blood and plasma samples. The red cell volumes of the mice were estimated from their body weights. The radioactivity of the infused red blood cells was

determined using formula (a); the radioactivity of the red blood cells in the post-infusion samples was calculated using formula (b). The posttransfusion red cell survival at each sampling time was calculated as follows:

$$(f) \text{ RED CELL SURVIVAL, \%} = \frac{\text{POST INFUSION RBC, cpm/ml} \times \text{RED CELL VOLUME, ml} \times 100}{\text{INFUSED RBC, cpm}}$$

The half-life (T50) following infusion of ^{51}Cr -radiolabeled fresh and 14-day-old liquid preserved heparinized red blood cells was measured. In each of 5 experiments, mice were infused with 0.25 ml of a ^{51}Cr -labeled red blood cell suspension. Blood samples were collected at 24 hours, 7 days, and 10 days following the infusion. Hematocrit was measured in the ^{51}Cr -labeled red blood cell suspension and the post-infusion samples. The blood samples were centrifuged at 2400 RPM (590 X g) for 6 to 8 minutes, and the supernatant or plasma was separated. Radioactivity was measured in the injectate samples and in the post-infusion blood and plasma samples. The T50 is reported as the time at which 50% of the radioactivity disappeared using logarithmic extrapolation.

Organ Blood Volumes

In each of 12 studies, 2 mice were sacrificed 1 hour following infusion: In one of the 2 mice, the liver, spleen, kidneys, heart, lungs, skeleton, skeletal muscles, skin, and gastrointestinal tract were isolated and weighed; in the second mouse a blood sample was collected. The blood volume was determined in each organ from the presence of radioactivity following the infusion of fresh ^{51}Cr -labeled red blood cells. Radioactivity was measured in the injectate, the supernatant of the injectate, and in the organs, blood, and plasma.

The measured blood radioactivity was corrected for the differences in total blood volume between the two mice. Total blood volume (TBV, ml) was estimated from body weight.

$$(g) \text{ CORRECTED BLOOD RADIOACTIVITY, cpm/ml} = \frac{\text{MEASURED BLOOD, cpm/ml} \times \text{TBV OF MOUSE WHICH PROVIDED THE BLOOD SAMPLES}}{\text{TBV OF MOUSE WHICH PROVIDED THE ORGANS}}$$

The blood volume of each organ was calculated per gram of organ weight using the radioactivity in the organ and the corrected radioactivity per milliliter of blood (g).

$$(h) \text{ ORGAN BLOOD VOLUME, ml/g} = \frac{\text{ORGAN, cpm}}{\text{BLOOD, cpm/ml} \times \text{ORGAN, g}}$$

The organ blood volumes were calculated for liver, spleen, kidney, heart, lungs, skeleton, skeletal muscle, skin and gastrointestinal tract, and were estimated from the body weights of the mice infused with the liquid preserved red blood cells.

The organs were not washed, and the radioactivity measurement in the organs included the blood adhering to the organ. In three studies, the radioactivity in the organs was measured 30 minutes following the infusion of fresh ^{51}Cr -labeled red cells. The organs were then washed with saline, and assays were made in the washed organs and the waste solutions for radioactivity.

The Distribution of Nonviable Red Blood Cells in Organs

In 8 experiments, measurements were made of the survival of liquid preserved syngeneic red blood cells and the distribution of ^{51}Cr -labeled red cells in the organs. In each experiment, 12 mice were infused with 0.25 ml of the ^{51}Cr -labeled red cell suspension. Six mice were sacrificed at one hour, and blood samples were obtained. The remaining 6 mice were sacrificed at 24 hours, and blood and organ

samples were obtained. The liver, spleen, kidneys, heart, and lungs were isolated and weighed, and blood samples were collected one hour and 24 hours after the infusion of the ^{51}Cr -labeled preserved red cells. In one experiment, samples were obtained only at the 24-hour post-infusion period. For the mice sacrificed at one hour, only the femurs were isolated, and for mice sacrificed 24 hours after infusion, the entire skeleton was isolated. The radioactivity was measured in each organ and in the post-infusion blood and plasma samples and in the ^{51}Cr -labeled injectate and supernatant of the injectate.

The radioactivity in the nonviable red cells sequestered in each organ was determined from the total organ radioactivity minus the estimated organ radioactivity associated with the viable circulating ^{51}Cr -labeled red cells. The radioactivity in the circulating viable red cells in each organ was determined from the blood radioactivity and the blood volume of the organ estimated from the weight of the organ. The blood radioactivity was adjusted to the differences in total blood volume in the mouse from which the organs were obtained and the mouse from which the blood samples were obtained, using the formula (g).

The radioactivity associated with circulating viable red cells (vRBC) in each organ was calculated as follows:

$$(i) \text{ ORGAN vRBC RADIOACTIVITY, cpm} = \text{BLOOD, cpm/ml} \times \text{ORGAN BLOOD VOLUME, ml}$$

The extravascular radioactivity associated with the sequestered nonviable red cells (nvRBC) in each organ was calculated from the

circulating viable red cell radioactivity (i) subtracted from total organ radioactivity:

$$(j) \text{ ORGAN nvRBC RADIOACTIVITY, cpm} = \text{ORGAN, cpm} - \text{vRBC, cpm}$$

The sequestered nonviable red cell radioactivity in the organ (j) was expressed as percentages of infused red blood cell radioactivity (a) and nonviable red cell radioactivity, and was related to the organ weight.

$$(k) \% \text{ SEQUESTERED RBC} = \frac{\text{ORGAN nvRBC, cpm}}{\text{INFUSED RBC, cpm}}$$

$$(l) \% \text{ SEQUESTERED NONVIABLE RBC} =$$

$$\frac{\text{ORGAN nvRBC, cpm} \times 100}{\text{INFUSED RBC, cpm} \times (100 - \text{SURVIVAL, \%})}$$

To estimate the weight of bone marrow in the femur, nine femurs were weighed before and after being cleaned of bone marrow by scraping. In the experiments in which the total femur was weighed, the mean percentage of the femur weight associated with bone marrow was used to calculate the uptake of the ^{51}Cr -labeled red blood cells by one gram of bone marrow. In one group of mice, the radioactivity in the femurs was found to be related to the radioactivity in the entire skeleton following the infusion of ^{51}Cr -labeled liquid preserved red blood cells. This relationship was used only in the experiments in which the femurs were isolated to estimate the ^{51}Cr uptake by the total skeleton.

The Distribution of Radioactivity Within the Femur

In 6 mice the distribution of radioactivity within the femurs was measured following the infusion of ^{51}Cr -labeled fresh red blood

cells and red blood cells stored at +4C in heparin for 15 days. Three mice in the control group were each infused with 0.25 ml of 0.9% saline solution containing 2 uCi/ml of ^{51}Cr . Each mouse was sacrificed 24 hours after infusion, and the femur was isolated and cleaned of muscle tissue. The right femur of each mouse was placed in a gamma tube and was used to measure total bone radioactivity. To remove all residual muscle and connective tissue from the left femur, the femur was incubated in 2% collagenase solution (Millipore Corp. Freehold, NJ. 126 u/mg) at 37°C for 30 minutes, washed with saline using a vortex mixer,, and incubated in 0.3% trypsin solution (Flow Laboratories, McLean, VA) at 37°C for 30 minutes. Both ends of the femur were severed and removed, and the bone marrow was flushed from the shaft with normal saline four times. The epiphysis and shaft were incubated again in a collagenase solution and a trypsin solution, flushed with saline, wrapped in gauze, and then suspended in 20 ml of formic acid-sodium citrate at 25C for 24 hours. The pieces of tissue were removed, washed in normal saline, and counted for radioactivity along with all the incubation and wash solutions collected at each step. The radioactivity in the decalcified bone matrix, the acid soluble calcium component, bone marrow, and extraosseus muscle and connective tissue was calculated as a percent of total femur radioactivity.

Total Recovery of Infused ^{51}Cr Radioactivity

Eight mice were infused with ^{51}Cr -labeled red blood cells obtained from blood that had been stored at +4C for 15 days. Urine and feces samples were collected during the 24-hour period following infusion. Blood samples from 5 of the mice were collected and pooled

24 hours following the infusion. Three of the mice were sacrificed 24 hours following infusion: the livers, spleens, kidneys, hearts, lungs, skeletons, skeletal muscles, skins, and gastrointestinal tracts were isolated. The radioactivity in each organ was counted, together with that in the ^{51}Cr -labeled injectate and blood samples and in the 24-hour urine and feces samples. The percentages of infused radioactivity recovered in each organ and in the blood, urine and feces samples were recorded.

RESULTS

⁵¹Cr-Labeled Red Cell Volume

The red cell volumes (RCV) of B6C3HF₁ mice, ranging from 0.70 to 0.93 ml, were correlated to body weights (BW) as follows: $RCV = 0.017 BW + 0.29$ ($r=0.9962$, $p<0.001$) (Table 1, Figure 1). Each of the 8 values reported represented a pooled sample from 3 mice. The relationship of total blood volume (TBV) to body weight was: $TBV = 0.041 BW + 0.595$ ($r=0.908$, $p<0.001$) (Table 1, Figure 2). The total body hematocrits reported in Table 1 were measured in 1.2 ml of blood obtained at the time of exsanguination.

⁵¹Cr-labeling of Fresh and Liquid Preserved Mouse Red Blood Cells and In Vitro Loss of Mouse Red Blood Cells During Storage at +4C and after ⁵¹Cr-labeling

The in vitro loss of mouse red blood cells during storage at +4C for 14 days and after ⁵¹Cr-labeling was 32%. The in vitro loss of mouse red blood cells during storage in heparin at +4C for up to 24 days and after ⁵¹Cr-labeling was as high as 73%. The in vitro hemolysis of fresh red blood cells during the ⁵¹Cr labeling procedures was 5%. The mean red cell associated ⁵¹Cr radioactivity in the washed radiolabeled red cell suspension stored at +4C in heparin for up to 24 days was 91% (SD 6.6%).

Hematocrit Measurements

The volume of blood collected and the sampling site had only a minimal effect on the hematocrit measurement. The hematocrit in the 1.2 ml blood sample obtained from the abdominal aorta, which was approximately 67% of the total blood volume, was similar to that in

the 0.2 ml blood sample from the abdominal aorta (Table 2). Total blood volume and plasma volume were calculated from the hematocrit of the 1.2 ml blood samples from the abdominal aorta. The hematocrit in the 0.2 ml blood sample from the inferior vena cava was slightly lower (1 V%) than that in the 0.2ml blood sample from the abdominal aorta.

Survival Of Syngeneic Fresh and Liquid Preserved Red Blood Cells

In 8 experiments, the mean 24-hour survival of fresh syngeneic red blood cells collected into heparin or ACD anticoagulant was 95.1% (SD 5.7%) (Table 3). The value decreased linearly as a function of the length of storage at 4C. The longer the red cells were stored at +4C, the greater the reduction in survival. The 24-hour posttransfusion survival of the red cells stored at +4C for 3 to 7 days ranged from 83 to 85%, with a mean of 84%; that of the red blood cells stored at +4C for 10 to 15 days ranged from 26 to 80%, with a mean of 50% (Table 3, Figure 2); and the 24-hour posttransfusion survival of red cells stored for 19 to 24 days ranged from 16 to 44%, with a mean of 35% (Table 3, Figure 3).

Fresh ^{51}Cr -labeled red blood cells had a mean 24-hour posttransfusion survival value of 89% and a half-life of 13, 17.5, and 14 days. Syngeneic red blood cells stored at +4C for 14 days had a mean 24-hour posttransfusion survival value of 34%, and a half-life (T50) of 8.5 and 9.3 days (Table 4).

Organ Uptake Of Nonviable Red Blood Cells

To determine the extravascular ^{51}Cr radioactivity associated with sequestration of red blood cells in each organ, the organ radioactivity associated with the circulating viable red cells was subtracted from the total organ radioactivity. The mean blood volume

per gram of organ was calculated from the radioactivity of circulating red blood cells (Table 5).

Organ weight and radioactivity were measured in nonwashed organs. When washing was employed, radioactivity in the liver, spleen, and kidney was reduced by less than 7%. The percentages of radioactivity washed from the liver, spleen, kidneys, and lungs were 3.2, 5.0, 1.6, and 6.8, respectively (Table 6).

Twenty-four (24) hours after transfusion of liquid preserved syngeneic red blood cells, 12.6% of the radioactivity associated with the non-viable red blood cells in the total skeleton was present in the femur (Table 7). This percentage is slightly less than the value of 17% reported in the study by Keene and Jandl (33) in which ^{51}Cr -labeled antibody-coated red blood cells were infused into the rat. The nonviable red blood cells were sequestered in the liver, spleen and skeleton during the 24-hour post-infusion period (Table 8, Figure 4). As the percentage of nonviable red blood cells in the transfused blood increased, the sequestration in these organs increased. The spleen sequestered the greatest percentage of nonviable red blood cells one hour after infusion; the percentage 24 hours after infusion was similar to that in the skeleton and slightly lower than that in the liver. Red blood cells that were stored at +4C for 15 to 24 days exhibited 2 percent infused radioactivity in the lungs one hour following infusion, and 24 hours following infusion the value was 5% to 8%.

The mean weight of the bone marrow was 17.1% that of the femur (Table 9), and this value was used in estimating the uptake of ^{51}Cr radioactivity per gram of bone marrow in the femur. The uptake of

nonviable red cells per gram of tissue was higher in the spleen than in the liver or the bone marrow (Table 10). The extravascular radioactivity in the liver, spleen, and skeleton was expressed as a percentage of ^{51}Cr infused with nonviable red blood cells (Table 11). One hour after the infusion of ^{51}Cr -labeled red blood cells stored at $+4^{\circ}\text{C}$ for 11 to 24 days, 40 to 49% of the nonviable red cells were sequestered in the spleen, while 19 to 44% was in the liver, and 12 to 34% in the skeleton. Twenty-four hours after the infusion of the syngeneic red blood cells stored at $+4^{\circ}\text{C}$ for 11 to 24 days, the mean percentage of nonviable red cells sequestered in the spleen was 25%, in the liver 31%, and in the skeleton 30%.

Distribution Of Radioactivity in the Femur

Twenty-four hours after the infusion of fresh or liquid-preserved ^{51}Cr -labeled red cells, the extraosseous tissue was removed and the distribution of radioactivity within the femur was measured. In the fresh ^{51}Cr -labeled red cells, 28% of the total radioactivity in the femur was found to be related to circulation of the red cells through the extraosseous muscle and connective tissue and 72% to circulation within the femur (Table 12). In the liquid preserved ^{51}Cr -labeled red blood cells, 4% of the total femur radioactivity in the femur was related to circulation through the extraosseous tissue, and 96% represented circulation within the femur suggesting a loss of nonviable red blood cells from the circulation and sequestration within the femur.

Fresh ^{51}Cr -labeled red blood cells showed 41% of the radioactivity in the marrow of the femur, 56% in the acid-soluble

calcium component (ASCC), and 4% in the decalcified matrix. In the ^{51}Cr -labeled liquid-preserved red blood cells, 61% of intraosseous radioactivity was in the marrow, and 39 percent was in the acid soluble calcium component (ASCC); no radioactivity was observed in the decalcified matrix.

Twenty-four hours after the infusion of ^{51}Cr saline, 11.5% of total femur radioactivity was located in the extraosseous tissue, suggesting in vivo radiolabeling of red blood cells. Within the femur, 87% of the intraosseous radioactivity was associated with the acid soluble calcium component (ASCC).

Recovery of Infused Radioactivity

Following the infusion of ^{51}Cr -labeled red blood cells stored in heparin at +4C for 15 days, approximately 100% of infused radioactivity was recovered (Table 13). In addition to the extravascular radioactivity found in the liver, spleen, skeleton, lungs, and kidneys, approximately 5% radioactivity was found in muscles, 3% in skin, and 2% in the gastrointestinal tract. The 24-hour excretion of ^{51}Cr was 15.8% in urine and 2.4% in feces. Following infusion of the ^{51}Cr saline, there was rapid removal of the radioactivity from the circulation: 38% was excreted into the urine and feces during the first 24-hours after infusion.

DISCUSSION

Fresh syngeneic red blood cells collected into either heparin or acid citrate dextrose (ACD) exhibited 24-hour posttransfusion survival values of 95%. When the syngeneic red blood cells were stored at +4C for as long as 24 days, 24-hour posttransfusion survivals were reduced. Red blood cells stored at +4C in heparin for 7 days had 24-hour posttransfusion survivals of 84%. After 10 to 15 days of storage at +4C in heparin or acid citrate dextrose, 24-hour posttransfusion survival values were only 50% of normal. When the red blood cells were stored in heparin at +4C for 19 to 24 days, 24-hour posttransfusion survival values were reduced to 35%.

Mouse red blood cells rendered non-viable during liquid preservation and labeled with ^{51}Cr before transfusion showed sequestration mainly in the liver, skeleton, and spleen. The greatest uptake of the ^{51}Cr -labeled non-viable red blood cells was seen in the spleen one hour following infusion and in the liver 24 hours following infusion. Sequestration of the non-viable syngeneic red blood cells was similar in the spleen and skeleton. The sequestration of non-viable syngeneic red blood cells per gram was greater in the spleen and bone marrow than in the liver.

Twenty-four hours after the infusion of ^{51}Cr -labeled red blood cells stored at +4C in heparin for 15 days, a small amount of ^{51}Cr radioactivity was sequestered in the skeletal muscle, skin, and gastrointestinal tract. Following distribution of the

^{51}Cr -labeled non-viable red blood cells in the RE system and release of the free ^{51}Cr , the free ^{51}Cr and ^{51}Cr bound to plasma protein distributed into the extravascular space. Studies in humans have shown that skin and muscle contain 50% of the extravascular volume.³⁸

Our studies suggest that the non-viable red blood cells were sequestered in the marrow portion of the bone and that free ^{51}Cr was incorporated within the acid-soluble calcium component of the bone. The site of destruction of the red blood cells is related to the source of the red cell injury and the species studied³⁰⁻³⁷.

We have concluded that syngeneic red blood cells rendered non-viable as a result of liquid preservation may produce immune suppression resulting from reticuloendothelial blockage.

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FIGURE 1

The relation between body weight and red cell volume measured using ^{51}Cr -labeled isologous red cells in mice.

Figure 1.

RELATION BETWEEN BODY WEIGHT AND RED CELL VOLUME OF THE MOUSE

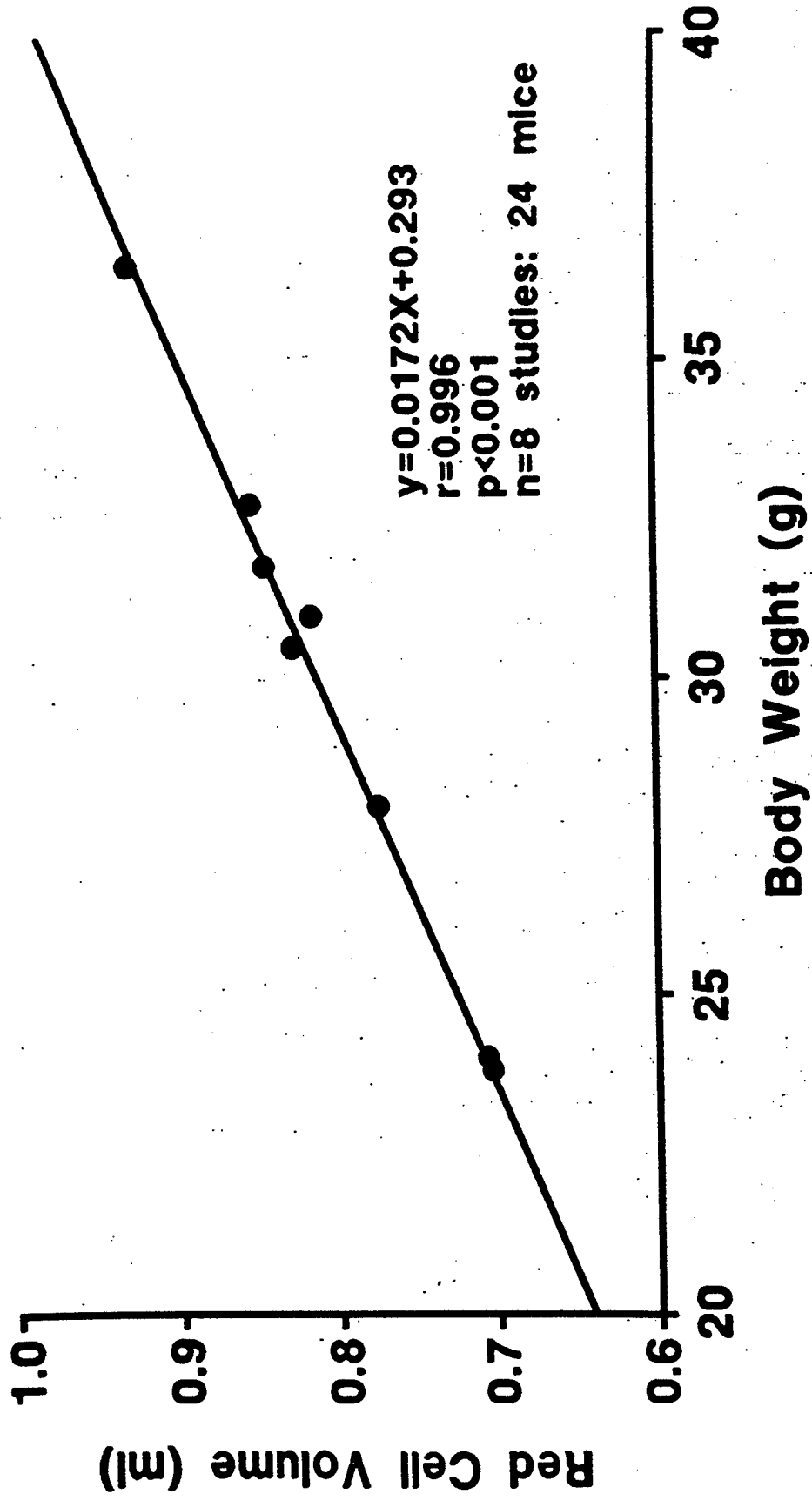


FIGURE 2

Relation between body weight and total blood volume of the mouse.

Figure 2.

RELATION BETWEEN BODY WEIGHT AND
TOTAL BLOOD VOLUME OF THE MOUSE

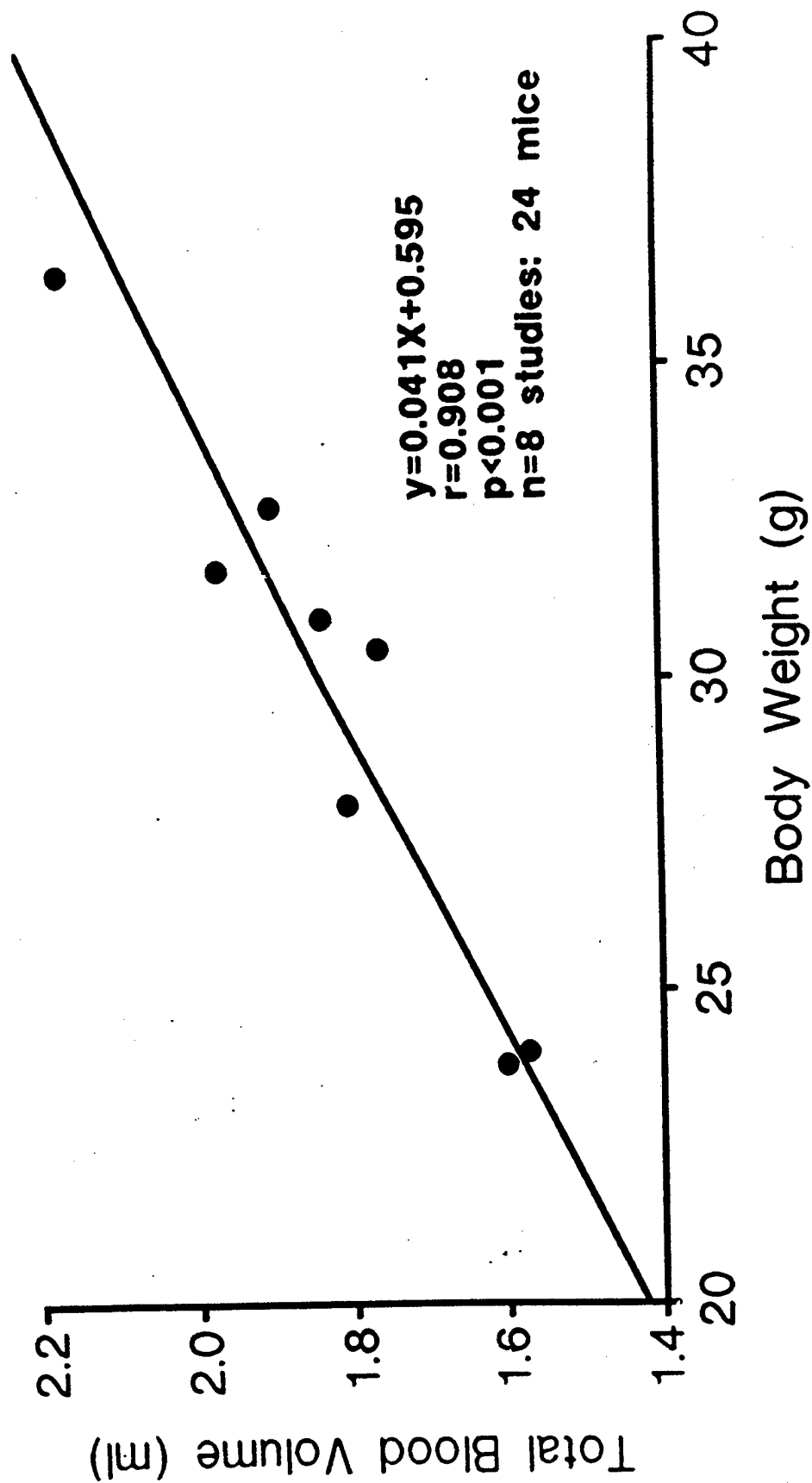


FIGURE 3

Relation between length of storage of mouse blood at +4C and the ^{51}Cr 24-hour posttransfusion survival values. The liquid preserved red blood cells were labeled with ^{51}Cr and the red blood cell volume of the recipient mouse was estimated from the weight of the mouse.

**RELATION BETWEEN 4C STORAGE AND 24 HR
POSTTRANSFUSION SURVIVAL OF MOUSE RED CELLS**

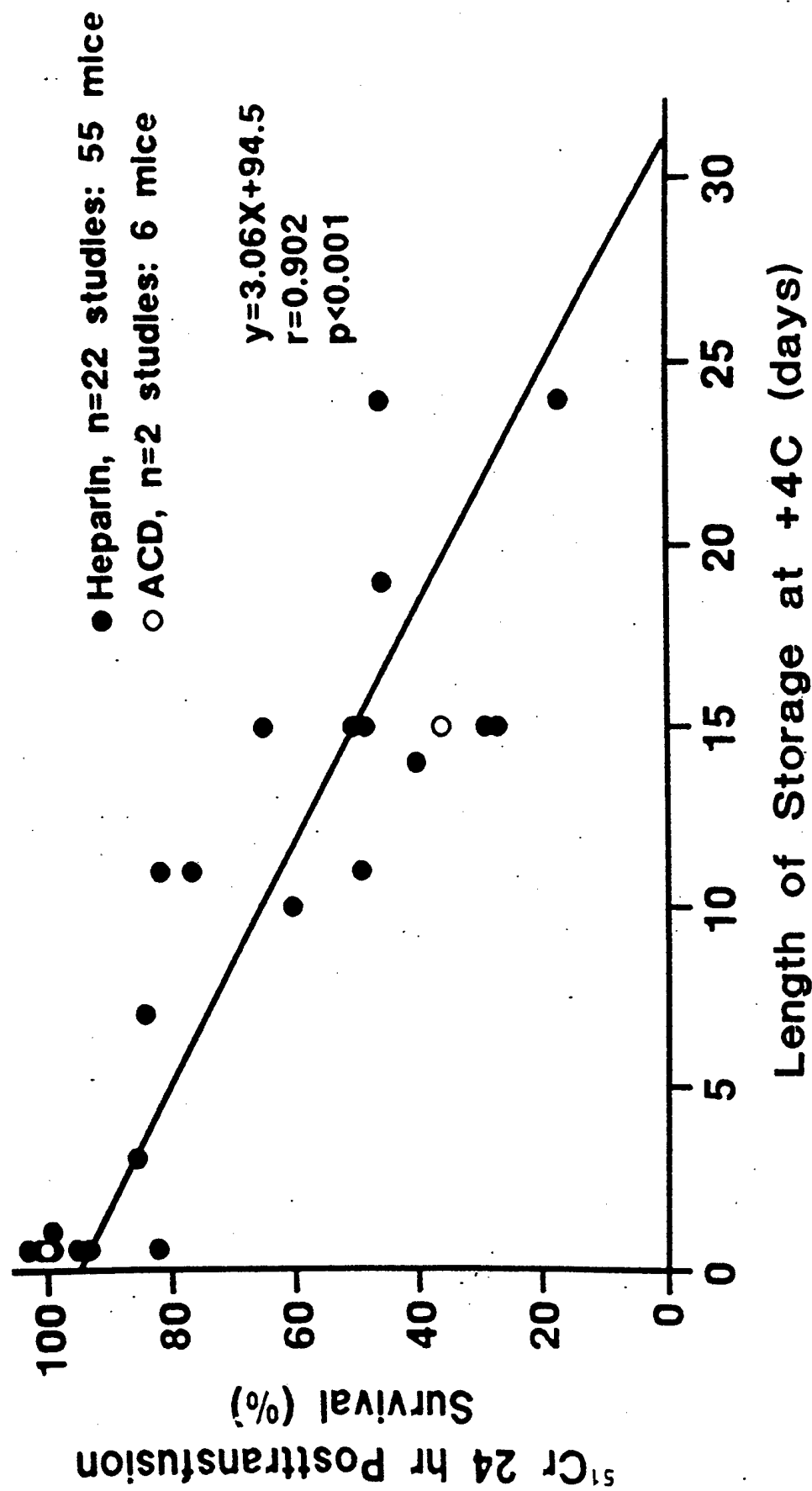


Figure 3

FIGURE 4

Sequestration of ^{51}Cr -labeled nonviable red blood cells in liver, spleen, and skeleton 24 hours following infusion. The red cells were infused after storage in heparin or ACD anticoagulant at 4C from 11 to 24 days.

DISTRIBUTION OF ⁵¹CR-LABELED NONVIABLE RBC 24hr AFTER TRANSFUSION

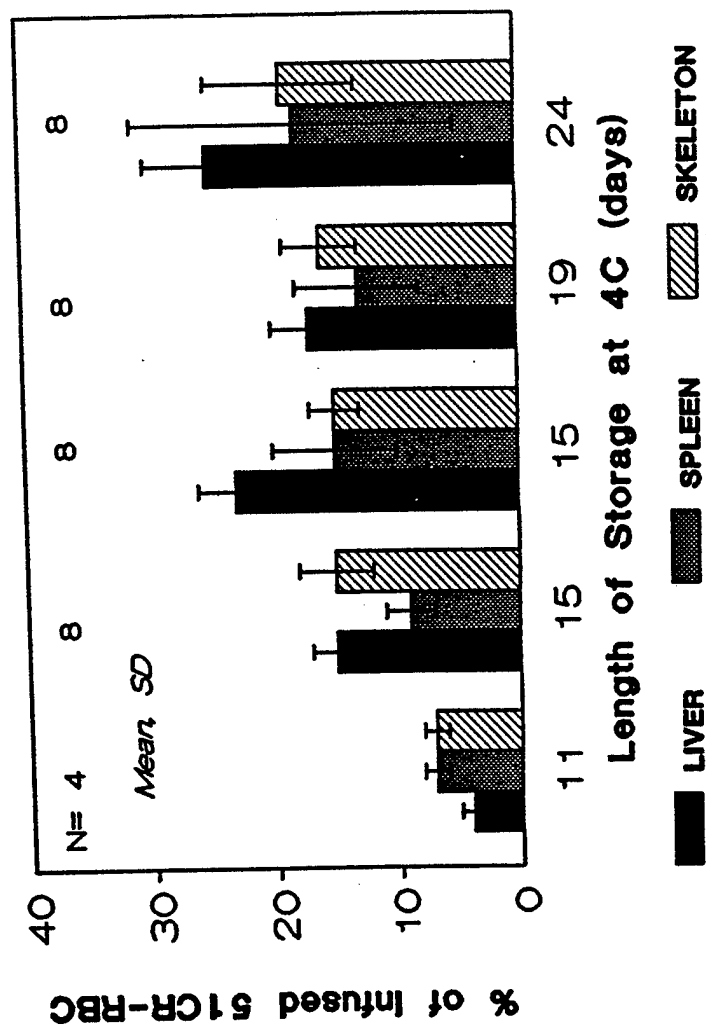


Figure 4

TABLE 1

BODY WEIGHT, HEMATOCRIT, 51CR RED CELL VOLUME, PLASMA VOLUME, AND
TOTAL BLOOD VOLUME OF THE MOUSE

POOLED BLOOD SAMPLE (# MICE/ POOL)	MEAN BODY WEIGHT (g)	TOTAL BODY HEMATOCRIT (%)	51CR RED CELL VOLUME (ML)	CALCULATED PLASMA VOLUME (ML)	TOTAL BLOOD VOLUME (ML)
3	23.8	44.0	0.703	0.895	1.598
3	24.0	45.0	0.706	0.863	1.569
3	28.0	43.0	0.773	1.025	1.798
3	30.5	47.0	0.825	0.930	1.755
3	31.0	44.5	0.813	1.014	1.827
3	31.8	43.0	0.842	1.116	1.958
3	32.8	45.0	0.850	1.039	1.889
<u>3</u>	<u>36.5</u>	<u>43.0</u>	<u>0.925</u>	<u>1.226</u>	<u>2.151</u>
Mean:3	29.8	44.3	0.805	1.014	1.818
SD:0	4.4	1.4	0.075	0.120	0.189
SEM:0	1.5	0.5	0.027	0.042	0.067
n:24	8	8	8	8	8

TABLE 2HEMATOCRIT VALUES OF 0.2 AND 1.2 ML SAMPLES OF HEPARINIZED BLOOD COLLECTED FROM THE ABDOMINAL AORTA AND INFERIOR VENA CAVAA. 1.2 ML BLOOD SAMPLE OBTAINED AFTER EXSANGUINATION

		<u>ABDOMINAL AORTA</u>	<u>INFERIOR VENA CAVA</u>
n = 3	Mean:	41.8	-
	SD:	0.8	

B. 0.2 ML BLOOD SAMPLE

n = 7	Mean:	41.2	40.2
	SD:	1.4	1.0

Paired t: 2.39
p= 0.054

TABLE 3

⁵¹CR POSTTRANSFUSION SURVIVAL OF MOUSE RED BLOOD CELLS STORED AT +4C IN
HEPARIN OR ACD ANTICOAGULANT

ANTICOAGULANT	LENGTH OF STORAGE AT +4C (DAYS)	51Cr RADIO- ACTIVITY ASSOCIATED WITH THE INFUSED RBC	51CR POSTTRANSFUSION SURVIVAL (%)		
			10 MINUTE	1 HOUR	24 HOURS
Heparin	0	85	--	--	98
Heparin	0	--	--	--	93
Heparin	0	93	--	--	95
Heparin	0	96	--	--	103
Heparin	0	--	--	--	95
Heparin	0	87	--	--	98
Heparin	0	--	--	--	82
ACD	0	<u>92</u>	--	--	<u>97</u>
Mean:	0	90.6			95.1
SD:		4.0			5.7
n:		5			8
Heparin	3	91	94	--	85
Heparin	<u>7</u>	<u>96</u>	<u>100</u>	--	<u>83</u>
Mean:	5.0	93.5	97.0		84.0
SD:	2.8	3.5	4.2		1.4
n:	2	2	2		2
Heparin	10	76	--	--	59
Heparin	11	96	98	--	48
Heparin	11	93	103	--	80
Heparin	11	99	--	91	75
Heparin	14	75	65	--	39
Heparin	15	94	82	--	63
Heparin	15	93	--	81	47
Heparin	15	97	95	78	49
Heparin	15	--	--	--	28
Heparin	15	--	--	--	26
ACD	<u>15</u>	<u>--</u>	<u>--</u>	<u>85</u>	<u>35</u>
Mean:	13.4	90.4	88.6	83.8	49.9
SD:	2.0	8.8	13.7	4.9	17.0
n:	11	8	5	4	11
Heparin	19	95	95	85	44
Heparin	24	93	90	68	16
Heparin	<u>24</u>	<u>91</u>	<u>76</u>	<u>71</u>	<u>44</u>
Mean:	22.3	93	87	74.7	34.7
SD:	2.4	1.6	8.0	7.4	13.2
n:	3	3	3	3	3

TABLE 4

THE 24 HOUR POSTTRANSFUSION SURVIVAL VALUE AND THE LIFESPAN (T50) OF MOUSE RED BLOOD CELLS MEASURED ON THE DAY OF COLLECTION AND AFTER STORAGE IN HEPARIN AT +4C FOR 2 WEEKS

		<u>24 HOUR POSTTRANSFUSION</u>	
		<u>SURVIVAL</u>	<u>T50, DAYS</u>
<u>A. FRESH MOUSE</u> <u>RED BLOOD CELLS</u>	1.	93%	13.0
	2.	87%	17.5
	3.	88%	14.0
<u>B. MOUSE RED BLOOD CELLS</u> <u>STORED IN HEPARIN AT +4C</u> <u>FOR 2 WEEKS</u>			
	1.	37%	8.5
	2.	30%	9.3

TABLE 5

BLOOD VOLUMES OF THE ORGAN MEASURED 1 HOUR FOLLOWING INFUSION OF ^{51}CR Labeled FRESH RED BLOOD CELLS

Mean $\pm$ SD	<u>BLOOD VOLUME</u>		
	<u>% OF INFUSED RADIOACTIVITY</u>	<u>(ml/g ORGAN WEIGHT)</u>	<u>n</u>
Muscle	19.2 $\pm$ 5.1	0.047 $\pm$ 0.012	4
Skeleton	12.5 $\pm$ 1.5	0.083 $\pm$ 0.014	8
Liver	5.1 $\pm$ 1.5	0.071 $\pm$ 0.015	12
Skin	4.8 $\pm$ 0.9	0.020 $\pm$ 0.004	4
GI Tract	3.2 $\pm$ 1.3	0.018 $\pm$ 0.005	4
Kidneys	2.6 $\pm$ 0.7	0.120 $\pm$ 0.030	12
Lungs	2.3 $\pm$ 0.4	0.340 $\pm$ 0.070	12
Spleen	2.0 $\pm$ 1.0	0.450 $\pm$ 0.160	12
Heart	0.3 $\pm$ 0.1	0.061 $\pm$ 0.009	12

TABLE 6

⁵¹CR RADIOACTIVITY IN THE ORGANS AND IN THE WASH SOLUTION 1 HOUR FOLLOWING INFUSION OF ⁵¹CR LABELED FRESH MICE RED BLOOD CELLS.

ORGAN RADIOACTIVITY FOLLOWING WASHING			WASH SOLUTION RADIOACTIVITY	% OF THE TOTAL RADIOACTIVITY IN THE WASH SOLUTION
(CPM)			(CPM)	(%)
N = 3				
LIVER	Mean:	4672	147	3.2
	SD:	1498	18	0.3
SPLEEN	Mean:	913	45	5.0
	SD:	121	8	0.8
KIDNEYS	Mean:	2898	48	1.6
	SD:	48	11	0.4
LUNGS	Mean:	1260	86	6.8
	SD:	86	5	0.4

TABLE 7

RATIO OF FEMUR TO TOTAL SKELETON RADIOACTIVITY ASSOCIATED WITH THE
REMOVAL OF NONVIABLE RED BLOOD CELLS 24 HOURS FOLLOWING TRANSFUSION
OF ⁵¹CR LIQUID PRESERVED RED BLOOD CELLS

STORAGE AT +4C

RATIO OF THE FEMUR
TO TOTAL SKELETON
⁵¹CR RADIOACTIVITY
MEAN ± SD

<u>(DAYS)</u>	<u>n</u>	
15	8	10.2 ± 1.5
15	4	15.4 ± 1.8
19	4	10.8 ± 1.4
24	8	14.4 ± 4.5
	24	Mean: 12.6 ± 2.5

TABLE 8

THE 51CR SURVIVAL OF LIQUID PRESERVED SYNGENEIC MICE RED BLOOD CELLS AND THE DISTRIBUTION OF NON VIABLE RED BLOOD CELLS STORED IN HEPARIN AND ACD ANTICOAGULANT AT +4C FOR 11 TO 24 DAYS

ANTICOAGULANT	STORAGE AT 4C DAYS		RED CELL SURVIVAL (%)	% OF INFUSED RADIOACTIVITY ASSOCIATED WITH THE NONVIABLE RBC				
				LIVER (%)	SPLEEN (%)	SKELETON (%)	LUNGS (%)	KIDNEYS (%)
<u>1 HOUR FOLLOWING INFUSION</u>								
Heparin	11	Mean:	91	2.3	2.3	0.6*	-0.1	-0.3
		SD:	--	0.8	0.7	1.2	0.1	0.3
		n:	1	4	4	4	4	4
Heparin	15	Mean:	80	3.3	6.2	3.9*	0.4	0.2
		SD:	2	1.0	2.2	2.3	0.7	0.3
		n:	2	8	8	8	8	8
ACD	15	Mean:	85	4.4	9.0	7.6*	1.5	-0.3
		SD:	--	0.8	1.2	0.9	0.4	0.1
		n:	2	8	8	8	8	8
Heparin	24	Mean:	70	8.9	18.9	9.5*	0.5	0.7
		SD:	2	1.0	1.4	2.0	0.5	0.5
		n:	2	8	8	8	8	8
<u>24 HOURS FOLLOWING INFUSION</u>								
Heparin	11	Mean:	75	3.7	6.7	6.6	-0.2	1.5
		SD:	--	1.0	1.0	0.9	0.4	0.3
		n:	1	4	4	4	4	4
Heparin	15	Mean:	48	15.4	8.9	14.7	-0.2	5.2
		SD:	1	2.4	2.2	2.7	0.2	1.4
		n:	2	8	8	8	8	8
ACD	15	Mean:	35	22.6	14.7	14.7	0.1	6.5
		SD:	--	2.8	4.5	2.3	0.1	1.6
		n:	1	4	4	4	4	4
Heparin	19	Mean:	44	16.5	13.1	15.9	-0.1	5.2
		SD:	--	2.9	5.3	2.7	0.1	1.7
		n:	1	4	4	4	4	4
Heparin	24	Mean:	30	25.2	18.1	19.4	-0.1	7.9
		SD:	20	4.9	12.8	6.4	0.3	2.1
		n:	2	8	8	8	8	8

*Value was estimated from the radioactivity in the femurs.

TABLE 9WEIGHTS OF FEMUR MARROW AND TOTAL FEMUR

FEMUR MARROW	FEMUR	% OF THE WEIGHT OF FEMUR MARROW TO THE WEIGHT OF THE FEMUR
(g)	(g)	
.0096	.0736	13.1
.0152	.0835	18.4
.0114	.0784	14.6
.0128	.0827	15.5
.0181	.0861	21.1
.0131	.0698	18.8
.0141	.0815	17.4
.0127	.0717	17.8
.0170	.1002	17.0
	Mean:	17.1
	SD:	2.4
	n:	9

TABLE 10

51CR RADIOACTIVITY ASSOCIATED WITH SYNGENEIC NON VIABLE LIQUID
PRESERVED MICE RED BLOOD CELLS IN THE LIVER, SPLEEN, AND BONE
MARROW OF THE FEMUR 24 HOURS AFTER TRANSFUSION

<u>ANTICOAGULANT</u>	<u>STORAGE</u>		<u>N</u>		<u>LIVER</u> (CPM/GM)	<u>SPLEEN</u> (CPM/GM)	<u>FEMORAL</u> <u>BONE MARROW</u> (CPM/GM)
	<u>AT +4C</u>	<u>(DAYS)</u>					
Heparin	11		4	Mean:	3	69	29
				SD:	1	13	12
Heparin	15		8	Mean:	12	136	95
				SD:	2	18	29
ACD	15		4	Mean:	16	202	92
				SD:	1	57	29
Heparin	19		4	Mean:	12	173	102
				SD:	1	33	22
Heparin	24		8	Mean:	18	199	161
				SD:	2	63	77

TABLE 11

PERCENTAGE OF THE ^{51}Cr RADIOACTIVITY ASSOCIATED WITH THE NON VIABLE SYNGENEIC MICE RED BLOOD CELLS IN THE LIVER, SPLEEN, AND SKELETON 1 HOUR AND 24 HOURS FOLLOWING THE TRANSFUSION OF LIQUID PRESERVED RED BLOOD CELLS STORED AT +4C IN HEPARIN OR ACD FOR 11 TO 24 DAYS

ANTICOAGULANT	STORAGE AT +4C (DAYS)	N	% OF NON VIABLE RED CELL RADIOACTIVITY LIVER (%)	SPLEEN (%)	SKELETON (%)
<u>1 HOUR FOLLOWING INFUSION</u>					
Heparin	11	4	44.1	43.5	12.2*
Heparin	15	8	23.4	43.1	26.6*
ACD	15	4	19.4	39.9	33.6*
Heparin	24	8	<u>23.0</u>	<u>48.8</u>	<u>24.6*</u>
		Mean:	27.5	43.8	24.3
		SD:	11.2	3.7	8.9
<u>24 HOURS FOLLOWING INFUSION</u>					
Heparin	11	4	19.0	35.3	34.5
Heparin	15	8	33.6	19.7	32.5
ACD	15	4	37.7	24.1	24.1
Heparin	19	4	31.1	24.4	30.5
Heparin	24	8	<u>34.5</u>	<u>23.3</u>	<u>27.0</u>
		Mean:	31.2	25.2	29.7
		SD:	7.4	5.7	4.5

* Value was estimated from the ^{51}Cr radioactivity in the femurs.

TABLE 12

DISTRIBUTION OF RADIOACTIVITY IN THE FEMUR AT 24 HOURS FOLLOWING THE INFUSION OF ^{51}Cr LABELED FRESH AND LIQUID PRESERVED RED BLOOD CELLS OR ^{51}Cr SALINE

	(MEAN)		(MEAN + SD)		(MEAN + SD)	
	% OF ^{51}Cr INFUSED RADIOACTIVITY		% OF TOTAL FEMUR RADIOACTIVITY		% OF INTRAOSSEOUS RADIOACTIVITY IN THE FEMUR	
	BLOOD	SKELETON	EXTRA-OSSEOUS	INTRA-OSSEOUS	MARROW	ASCC* DECALCIFIED MATRIX
<u>INFUSATE</u>						
1. ^{51}Cr Fresh RBC n = 3	86	12	27.8 ± 3.4	72.2 ± 3.4	40.7 ± 1.7	55.7 ± 5.1 3.6 ± 1.1
2. ^{51}Cr RBC, in heparin stored at +4C for 4 days	32	19	4.3 ± 0.6	95.7 ± 0.6	60.7 ± 9.8	39.3 ± 10.4 0 -
3. ^{51}Cr Saline, n = 3	8	--	11.5 ± 2.6	88.5 ± 2.6	± 1.6 ± 0.6	87.3 ± 3.0 1.0 ± 0.3

* ASCC: Acid soluble calcium component

TABLE 13

RECOVERY OF RADIOACTIVITY 24 HOURS FOLLOWING INFUSION OF ⁵¹CR LABELED
LIQUID-PRESERVED RED BLOOD CELLS STORED AT +4C FOR 15 DAYS AND ⁵¹CR SALINE

% OF INFUSED RADIOACTIVITY

	<u>⁵¹CR SALINE</u>		<u>⁵¹CR RED BLOOD CELLS STORED IN HEPARIN AT +4C FOR 15 DAYS</u>	
	n	Mean	n	Mean
Blood	2	8.4	1	25.9
Organs	8	35.5	3	59.0
24 Hour Urine	2	28.9	1	15.8
24 Hour Feces	2	8.6	1	2.4
Total		81.4		103.1