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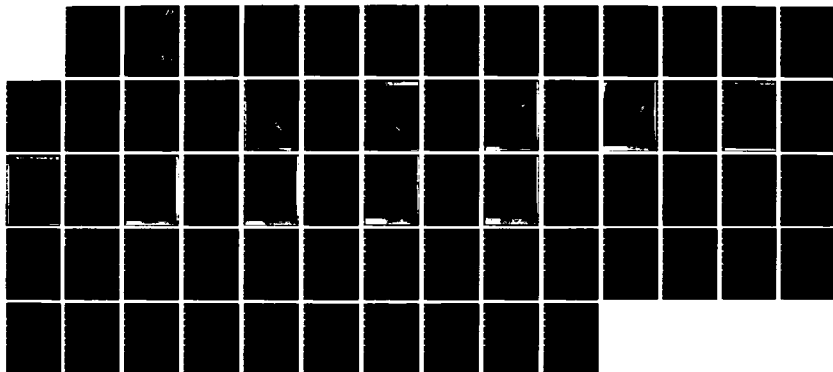
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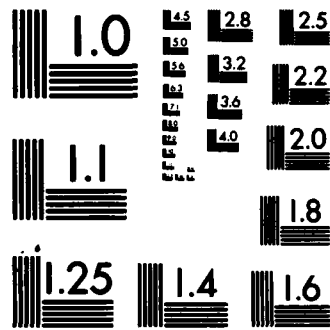
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TECHNICAL REPORT NO. 82-17

A COMPARISON OF METHODS USED FOR DETERMINATION OF THE 100% SURVIVAL
OF PRESERVED RED BLOOD CELLS

by

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When less than 20% of the transfused red blood cells were nonviable, i.e., 24-hour posttransfusion survival values of between 80-100%, the three methods gave comparable results. However, when 20 to 35% of the transfused red blood cells were nonviable, i.e., 24-hour posttransfusion survivals of 65 to 79%, or when greater than 35% were nonviable, 24-hour posttransfusion survival values of less than 65%, the ¹²⁵I albumin plasma volume method and the body surface area method gave lower values (about 5% p<0.001 and 10% p<0.001 respectively) than those obtained by the extrapolation method which gave an overestimated value.

These data indicate that when nonviable red blood cells are rapidly removed from the circulation immediately after transfusion, the extrapolation method gives an underestimation of the 100% survival value, resulting in an overestimation of the 24-hour posttransfusion survival value.

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ABSTRACT

↓ Three methods were evaluated to determine which gave the most accurate 100% survival value from which to estimate the 24-hour posttransfusion survival of ^{51}Cr -labeled preserved red blood cells: (1) indirect measurement of the recipient's red blood cell volume using ^{125}I albumin and the total body hematocrit; (2) estimation of the recipient's red blood cell volume from the body surface area nomogram; and (3) extrapolation of the radioactivity associated with the red blood cells in the recipient's circulation after the transfusion.

When less than 20% of the transfused red blood cells were nonviable, i.e., 24-hour posttransfusion survival values of between 80-100%, the three methods gave comparable results. However, when 20 to 35% of the transfused red blood cells were nonviable, i.e., 24-hour posttransfusion survivals of 65 to 79%, or when greater than 35% were nonviable, 24-hour posttransfusion survival values of less than 65%, the ^{125}I albumin plasma volume method and the body surface area method gave lower values (about 5% $p < 0.001$ and 10% $p < 0.001$ respectively) than those obtained by the extrapolation method which gave an overestimated value.

↓ These data indicate that when nonviable red blood cells are rapidly removed from the circulation immediately after transfusion, the extrapolation method gives an underestimation of the 100% survival value, resulting in an overestimation of the 24-hour posttransfusion survival value. ↗

INTRODUCTION

The 24-hour posttransfusion survival value is the principal indicator of the quality of preserved red blood cells, and to arrive at this value it is necessary to know the 100% survival of the transfused red blood cells. It is important to remember when calculating the 100% survival value that this measurement is accurate only when it includes all of the nonviable red blood cells in the transfused unit. Strumia and associates have cautioned against using the circulation of the ^{51}Cr -labeled preserved red blood cells as the sole measurement for determining the 100% survival value because nonviable red blood cells that may be removed from the circulation immediately after infusion are not represented in this measurement.¹⁻³ The ^{51}Cr labeling method used in conjunction with another method that measures the recipient's red blood cell volume gives a more accurate indication of the 100% survival value.⁴⁻¹² Several investigators have been using a relatively simple method of measuring the 100% survival by obtaining blood samples after the infusion of ^{51}Cr -labeled preserved red blood cells and extrapolating the radioactivity to time zero by a linear or logarithmic regression analysis.¹³⁻²¹

Data were collected at the Naval Blood Research Laboratory between 1973 and 1981 on three methods of measuring the 24-hour posttransfusion survival values of ten ml aliquots of ^{51}Cr -labeled preserved red blood cells autotransfused to 397 normal volunteers.²²⁻²⁵ The three methods used to determine the 100% survival values of the preserved red blood

cells were: (1) measurements of the radioactivity associated with the ^{51}Cr -labeled preserved red blood cells and of the recipient's red blood cell volume from the ^{125}I albumin plasma volume and the total body hematocrit;²⁶ (2) measurement of the radioactivity associated with the ^{51}Cr -labeled red blood cells and estimate of the recipient's red blood cell volume from the body surface area; and (3) linear or logarithmic extrapolation of the radioactivity associated with the ^{51}Cr -labeled red blood cells. A comparison of the results of these three methods is reported.

MATERIALS AND METHODS

The subjects studied between June 1973 and November 1981 were 371 healthy male volunteers and 26 healthy female volunteers from 21 to 65 years of age. From each donor, a 450 ml volume of blood was collected in 63 ml of citrate-phosphate-dextrose (CPD), citrate-phosphate-dextrose supplemented with an additional 25% glucose and 17.3 mg adenine (0.25 mM final blood concentration) (CPDA-1), citrate-phosphate-dextrose supplemented with an additional 75% glucose and 34.6 mg adenine (0.5 mM final blood concentration) (CPDA-2), or citrate-phosphate-dextrose supplemented with an additional 100% glucose and 34.6 mg adenine (0.5 mM final blood concentration) (CPDA-3).

Nonwashed and Washed Liquid-Stored Red Blood Cells

Three of these units were stored as whole blood at 4 C either in CPD for as long as 21 days or in CPDA-1 for as long as 35 days, and were not washed before transfusion. Four units were prepared as red blood cell concentrates with hematocrit values of 75 to 90 V% within 4 to 8 hours of blood collection and were stored at 4 C either in CPD for up to 21 days or in CPDA-1, CPDA-2 or CPDA-3 for up to 35 days; these units were washed with sodium chloride solutions and stored at 4 C for 24 hours before transfusion.

Rejuvenated Washed Red Blood Cell Concentrates

Ten units of red blood cell concentrates were rejuvenated after storage at 4 C in CPD for 22 to 25 days or in CPDA-1 for 25 to 38 days. Each unit was biochemically treated with 50 ml of a solution containing

100 mM/l pyruvate, 100 mM/l inosine, 100 mM/l glucose, 100 mM/l phosphate, and 5 mM/l adenine (PIGPA) or with 100 mM/l pyruvate, 100 mM/l inosine, 100 mM/l phosphate and 5 mM/l adenine (PIPA). The rejuvenated red blood cells were washed and stored at 4 C for as long as 3 days prior to transfusion.

Nonrejuvenated Cryopreserved Washed Red Blood Cell Concentrates

Eighty-one units of red blood cell concentrates with hematocrit values of 75 to 90 V% were cryopreserved after 4 C storage in CPD, CPDA-1, CPDA-2 or CPDA-3 for 3 to 8 days. Eleven of these units were frozen with 20% W/V glycerol and storage at -150 C in special polyolefin plastic bags. Seventy units were frozen with 40% W/V glycerol and storage at -80 C in special polyvinylchloride plastic bags, special polyolefin plastic bags, or in a 600 ml or 800 ml primary bag of a polyvinylchloride plastic multiple bag collection system. The glycerol cryoprotectant was removed from the red blood cells after thawing by washing with a sodium chloride-glucose-phosphate solution, and the washed red blood cells were stored in the resuspension medium at 4 C for as long as 3 days prior to transfusion.

Indated-Rejuvenated Cryopreserved Washed Red Blood Cell Concentrates

After storage in CPD for 3 to 8 days with hematocrit values of 75 to 90 V%, 100 units of red blood cell concentrates were biochemically treated with PIGPA or PIPA solution and then cryopreserved. Six of the 100 rejuvenated units were frozen with 20% W/V glycerol and storage at -150 C in special polyolefin plastic bags. The other 94 units were frozen with 40% W/V glycerol and storage at -80 C in special polyolefin

plastic bags, special polyvinylchloride plastic bags, or in the 600 ml or 800 ml primary bag of a polyvinylchloride plastic multiple-bag collection system. After thawing, all the units were washed with sodium chloride-glucose-phosphate solution and were then stored in the resuspension medium at 4 C for as long as 3 days prior to transfusion.

Outdated-Rejuvenated Cryopreserved Washed Red Blood Cell Concentrates

After storage in CPD for 22 to 26 days, in CPDA-1 for 36 to 38 days, or in CPDA-2 or CPDA-3 for 43 to 46 days with hematocrit values of 75 to 90 V%, 199 units of outdated red blood cells were biochemically treated with PIGPA or PIPA solution. Six of these units of outdated-rejuvenated red blood cells were frozen with 20% W/V glycerol and storage at -150 C in special polyolefin plastic bags; 193 units were frozen with 40% W/V glycerol and storage at -80 C either in special polyolefin plastic bags or in the 600 ml or 800 ml primary bag of a polyvinylchloride plastic multiple-bag collection system. After thawing, all the units were washed with a sodium chloride-glucose-phosphate solution and were then stored in the resuspension medium at 4 C for as long as 3 days.

^{51}Cr Labeling and Autotransfusion

A 20 to 30 ml volume of a red cell suspension from units of red blood cells prepared in the various ways described above was labeled with ^{51}Cr disodium chromate, and a 10 ml aliquot, containing 0.5 microcuries of ^{51}Cr , was autotransfused to the healthy volunteer simultaneously with 0.5 microcuries of ^{125}I -labeled albumin.²⁶

Blood samples were obtained prior to the infusions, 10 and 20 minutes or 15 and 30 minutes after infusions, and 24 and 72 hours and

7 days after infusions. The weight and height of each subject were measured on the day of study to estimate the body surface area.²⁷ The body surface area multiplied by a factor of 1020 was used to determine the theoretical red blood cell volume for the males and a factor of 806 for the females. Plasma volume also was determined from the total ^{125}I albumin injected radioactivity and the ^{125}I albumin radioactivity in the 10- or 15-minute blood sample. The recipient's red blood cell volume was calculated from the ^{125}I albumin plasma volume and the total body hematocrit (peripheral venous hematocrit multiplied by 0.89).²⁶

RESULTS

A comparison of the three methods used to calculate the 100% survival value showed that when 35% or more of the transfused red blood cells were nonviable, the linear extrapolation method did not give an accurate 100% survival value and overestimated the 24-hour posttransfusion survival value (Figure 1). This overestimation was seen in blood samples obtained 10 and 20 minutes after infusion and in samples obtained 15 and 30 minutes after infusion (Figures 2 and 3). The 100% survival values and the corresponding posttransfusion survival values were similar with the body surface area method and the measured ^{125}I albumin method when 35% of the transfused red blood cells were nonviable (Figure 4).

The 100% survival values obtained by the ^{125}I albumin method gave the most accurate estimation of 24-hour survival values regardless of the percentage of nonviable red blood cells in the transfused unit. When the percentage of nonviable red blood cells was about 15% the 24-hour posttransfusion survival value was less than 65%; when the percentage of nonviable red blood cells was 8% the 24-hour posttransfusion survival value was between 65 and 79%; and when the percentage was 3% the value was greater than 80% (Figure 5).

The extrapolated method, on the other hand, was accurate only when the percentage of nonviable red blood cells removed from the circulation during the 10- to 15-minute posttransfusion period was less than 20% (Figures 6 and 7). The overestimation in the 24-hour posttransfusion survival seen with the rapid loss of nonviable red blood cells was some-

times as high as 20% (Figure 8).

Frequency distribution plots were calculated to see whether the differences in 24-hour posttransfusion survival values were distributed in a symmetrical or asymmetrical pattern. A symmetrical pattern was seen in values obtained with the body surface area method and the ^{125}I albumin method (Figure 9). A symmetrical pattern also was observed in values obtained with the ^{125}I albumin method and the linear extrapolated method when 24-hour posttransfusion survival values were 80 to 100% (Figure 10).

In 107 studies in which 24-hour ^{51}Cr survival values were 65 to 79%, the values obtained by the linear extrapolation method were only slightly overestimated compared with values obtained with the ^{125}I albumin method (Figure 10). However, in 20 studies in which 24-hour ^{51}Cr survival values were less than 65%, the 24-hour posttransfusion survival values obtained by the linear extrapolation method were significantly overestimated compared with values obtained with the ^{125}I albumin method (Figure 10).

DISCUSSION

Red blood cells are transfused primarily to increase the patient's red blood cell volume, and the red blood cell transfusion is considered to be therapeutically effective if the 24-hour posttransfusion survival value is at least 70%. An accurate determination of 24-hour posttransfusion survival can be made only if an accurate 100% survival value has been calculated, and the calculation of 100% survival will be accurate only if it includes the percentage of nonviable red blood cells in the transfused unit that are fated for rapid removal from the recipient's circulation within the first 24 hours after transfusion.

Several methods have been used to determine the number of nonviable red blood cells in a transfused unit. The practice recommended by Strumia and by others¹⁻¹² is to estimate the 100% survival value by measuring separately the survival of the preserved red blood cells and the red blood cell volume of the recipient. This is done by labeling the preserved red blood cells with ^{51}Cr before transfusion and measuring the radioactivity associated with the red blood cells and simultaneously measuring the recipient's red blood cell volume either directly with ^{32}P or indirectly from measurements of plasma volume with ^{125}I albumin or Evans blue and total body hematocrit. Some investigators contend that it is not necessary to make an independent measurement of the recipient's red blood cell volume to obtain an accurate 24-hour posttransfusion survival value, and suggest that an accurate estimate of the 100% survival value can be obtained by constructing a linear or logarithm

extrapolated regression plot of the red blood cell-associated radioactivity;¹³⁻²¹ in our study the linear and logarithm extrapolations gave similar results. The practice recommended is to analyze blood samples obtained at specific intervals after the infusion of the ⁵¹Cr-labeled preserved red blood cells.

In 386 autotransfusion studies in which the body surface area method and the ¹²⁵I albumin method were used to calculate the 100% survival values, the 24-hour posttransfusion survival values were similar, with a symmetrical frequency distribution pattern.

Comparison of the linear/logarithm extrapolation method with the ¹²⁵I albumin method revealed similar 24-hour posttransfusion survival values as long as there were no more than 20% nonviable red blood cells in the transfusion. However, when the transfused red blood cells contained 20 to 35% nonviable red blood cells, the extrapolated 100% survival value overestimated the 24-hour posttransfusion survival by about 5%. When there were greater than 35% nonviable red blood cells, the overestimation was about 10%.

It is a relatively simple process to estimate the 100% survival value of preserved red blood cells by extrapolation of the radioactivity associated with the red blood cells in the circulation immediately after transfusion, but this method is accurate only when the number of nonviable red blood cells in the donor unit does not exceed 20%. The ¹²⁵I albumin method, on the other hand, gives an accurate estimate of the plasma volume when healthy recipients are studied but in patients gives an overestimation of the plasma volume and the red blood cell volume.^{28,29}

In our experiments in healthy volunteers, we were able to obtain an accurate determination of the 24-hour posttransfusion survival value of donor red blood cells by making independent measurements of the donor red blood cells by ^{51}Cr labeling and of the healthy recipient's red blood cell volume by ^{125}I albumin plasma volume and the total body hematocrit.

Much research effort has gone into ensuring that donor red blood cells have 24-hour posttransfusion survival values of greater than 70%, and this is an important quality control standard. The increase in red blood cell volume following transfusion produces an increase in the oxygen carriage of the arterial blood, and circulating preserved red blood cells increase both the plasma volume and total blood volume.²⁹ However, there is another quality control standard that should be initiated, and that is to ensure that donor red blood cells have normal oxygen transport function at the time of transfusion. Satisfactory oxygen transport function is especially important in anemic patients with fixed cerebral and coronary blood flow.³⁰ Determination of oxygen transport function can be made easily from in vitro measurements of red cell 2,3 DPG and does not require sensitive in vivo evaluation such as is needed for measurements of posttransfusion survival.

FIGURE 1.

Comparison of ^{51}Cr 24-hour posttransfusion survival values obtained by: estimation from the linear extrapolated 100% value using 10- and 20-minute post-infusion samples and 15- and 30-minute post-infusion samples; and measurement of ^{125}I albumin 100% survival value, in 397 autotransfusion studies.

FIGURE 2

Comparison of ^{51}Cr 24-hour posttransfusion survival values obtained by: estimation from the linear extrapolated 100% value using only the 10- and 20-minute post-infusion samples; and measurement of ^{125}I albumin 100% survival value, in 174 autotransfusion studies.

FIGURE 2
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⁵¹Cr 24 Hour Posttransfusion Survival
Sampled 10 and 20 Minutes After Transfusion

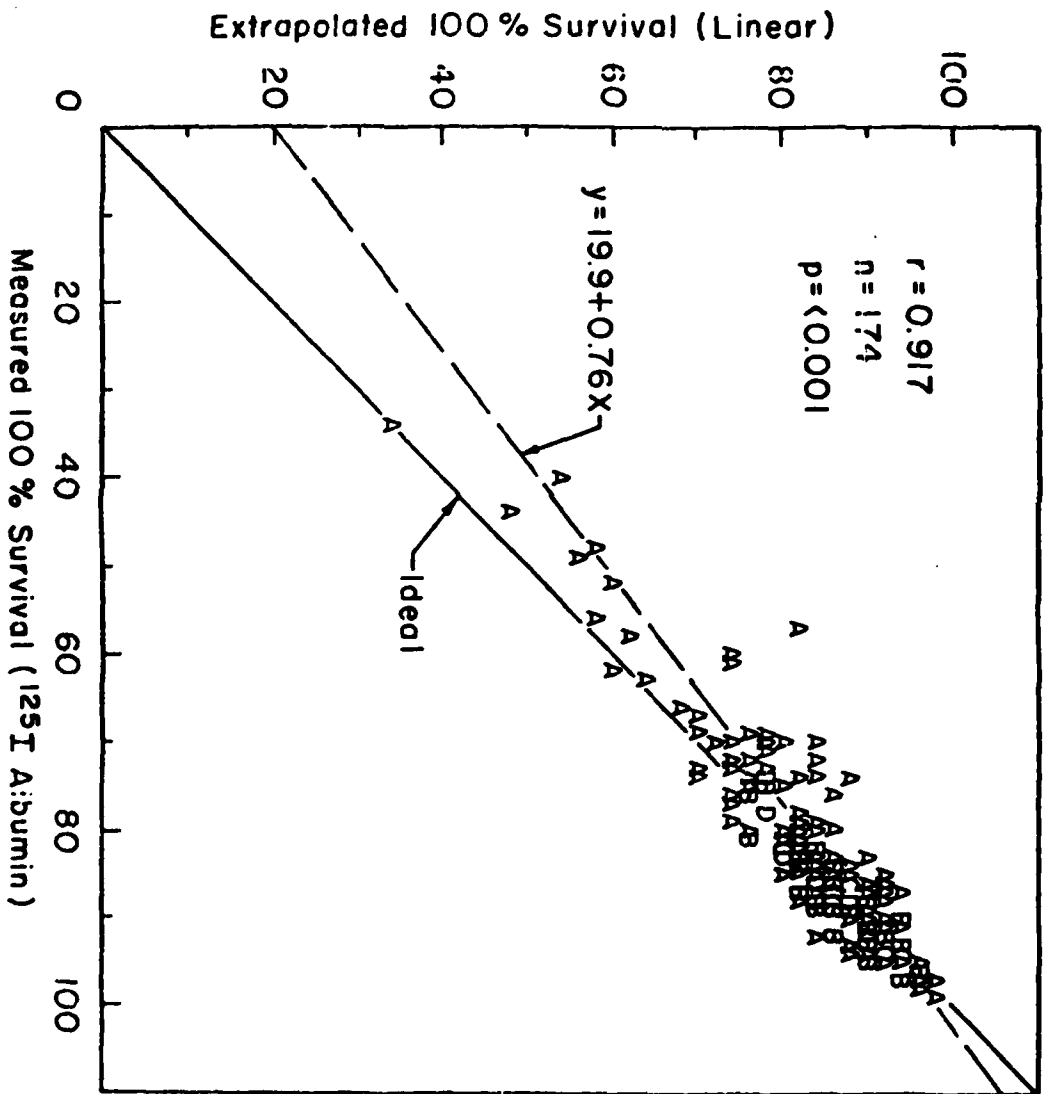


FIGURE 3

The relation between the ^{51}Cr 24-hour posttransfusion survival values of 223 autotransfusions estimated from the linear extrapolated 100% value using only the 15- and 30-minute post-infusion samples and the measured ^{125}I albumin 100% survival value.

FIGURE 3
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⁵¹Cr 24 Hour Posttransfusion Survival
Sampled 15 and 30 Minutes After Transfusion

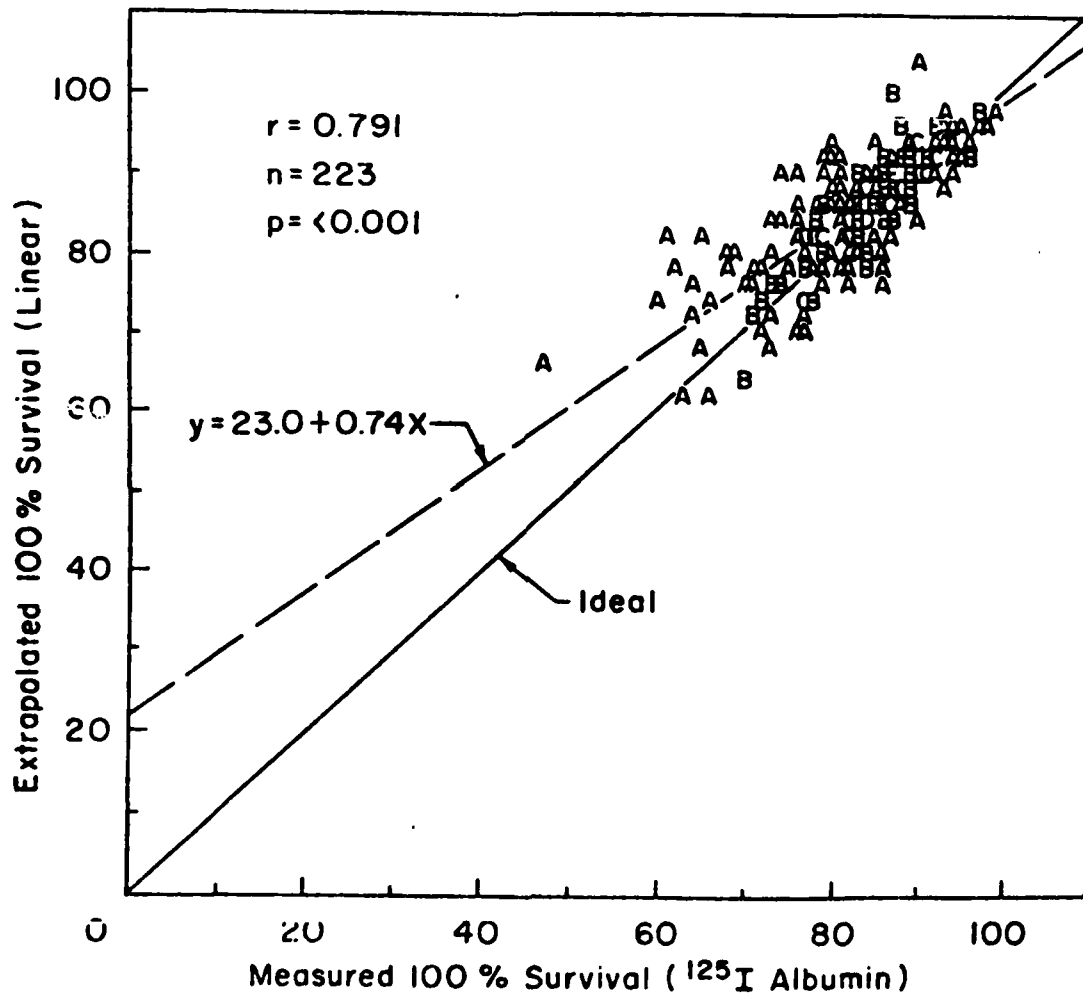


FIGURE 4

The relation between the ^{51}Cr 24-hour posttransfusion survival values of 386 autotransfusions estimated from the 100% survival value derived from the body surface area (BSA) and the measured ^{125}I albumin 100% survival value.

FIGURE 4

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⁵¹Cr 24 Hour Posttransfusion Survival

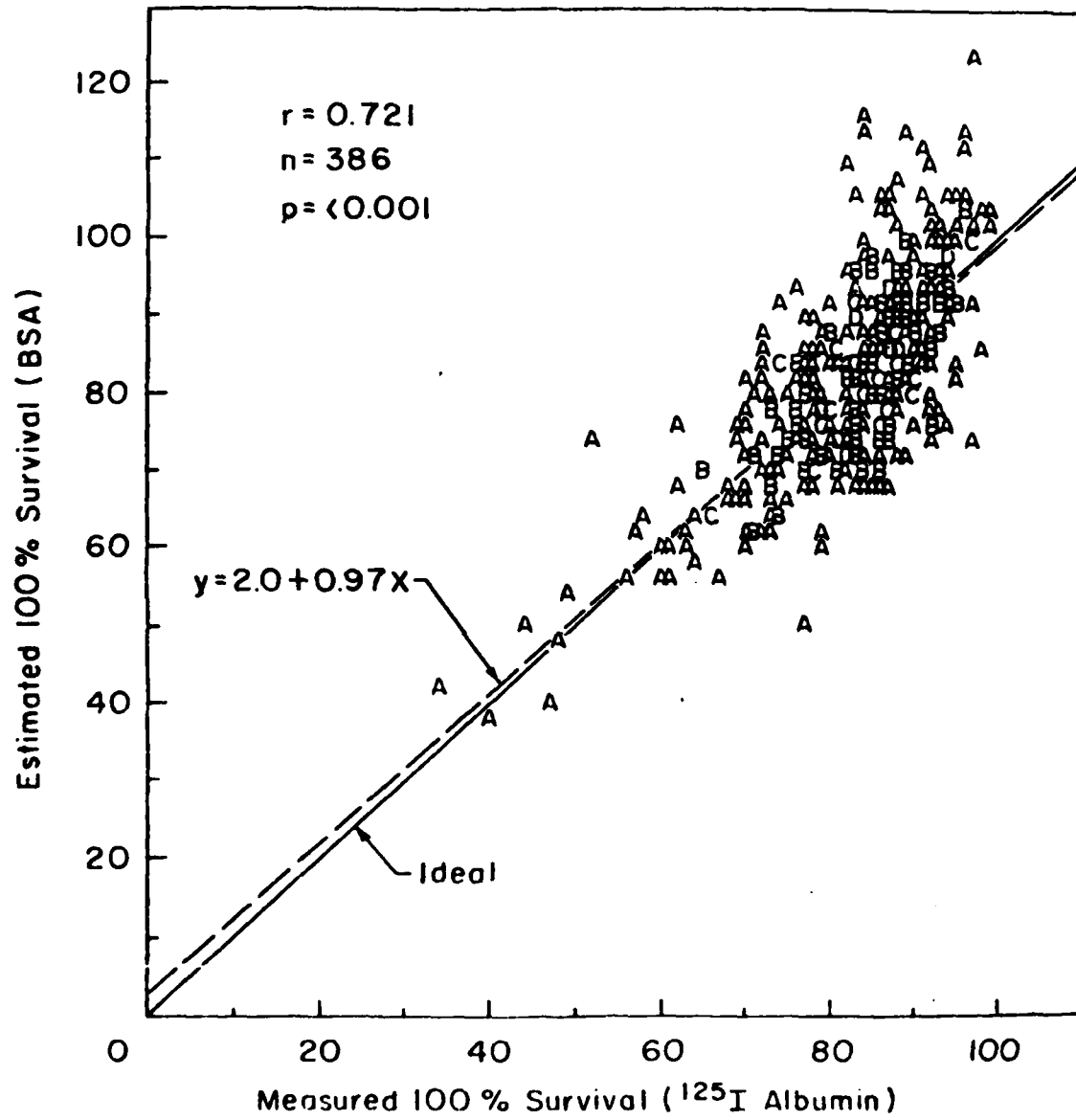


FIGURE 5

The initial loss of nonviable red blood cells during the 10- and 15-minute post-infusion period. The nonviable red blood cells were determined from the measured ^{125}I albumin 100% survival value when the 24-hour posttransfusion survival values were 80-100%, 65-79%, and less than 65%.

FIGURE 5

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Initial Loss of ^{51}Cr 10-15 Minutes after Infusion

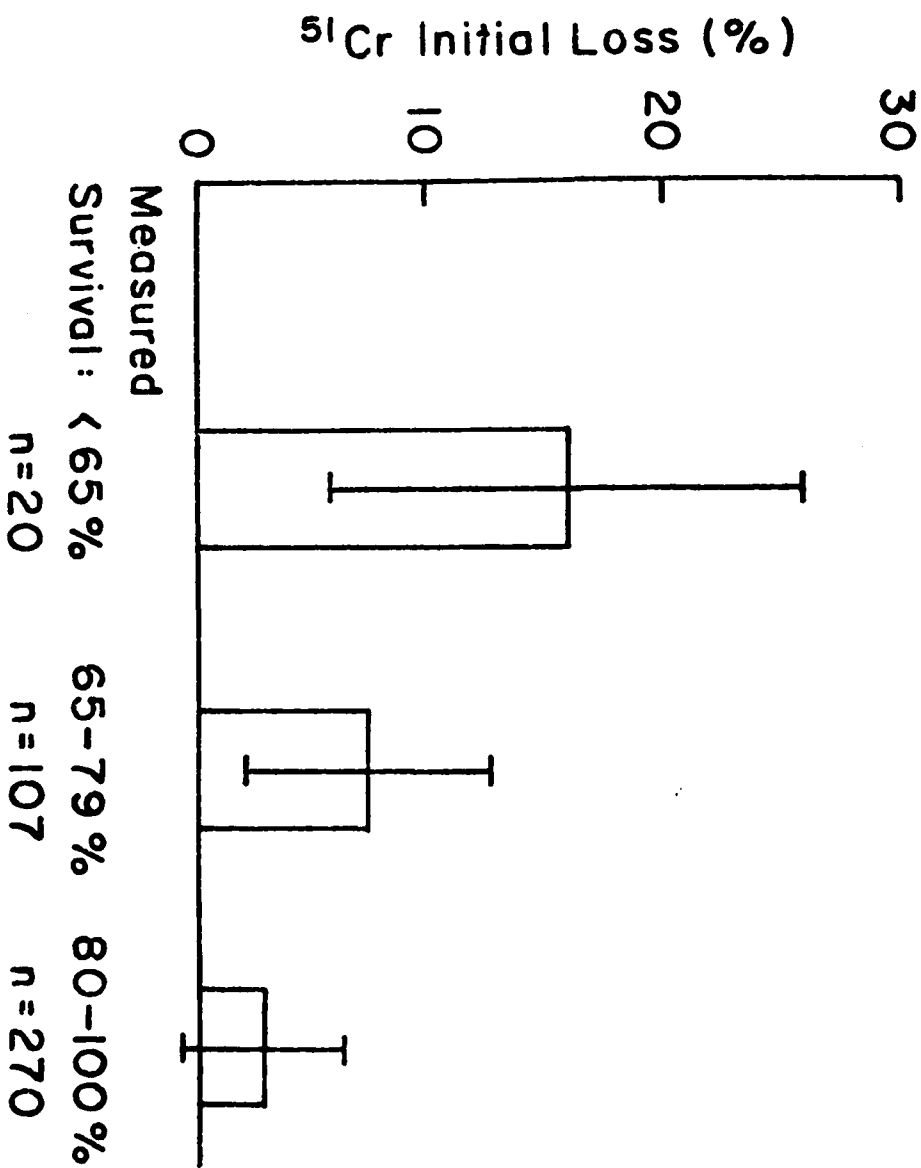


FIGURE 6

The 24-hour ^{51}Cr posttransfusion survival values estimated from the measured ^{125}I albumin 100% value; the 100% value estimated from the body surface area (BSA); the extrapolated linear 100% value; and the extrapolated logarithm (log) 100% value. The four methods to estimate the 100% survival value were compared when the 24-hour posttransfusion survival values measured using ^{125}I albumin 100% survival values were 80-100%, 65-79%, and less than 65%.

FIGURE 6

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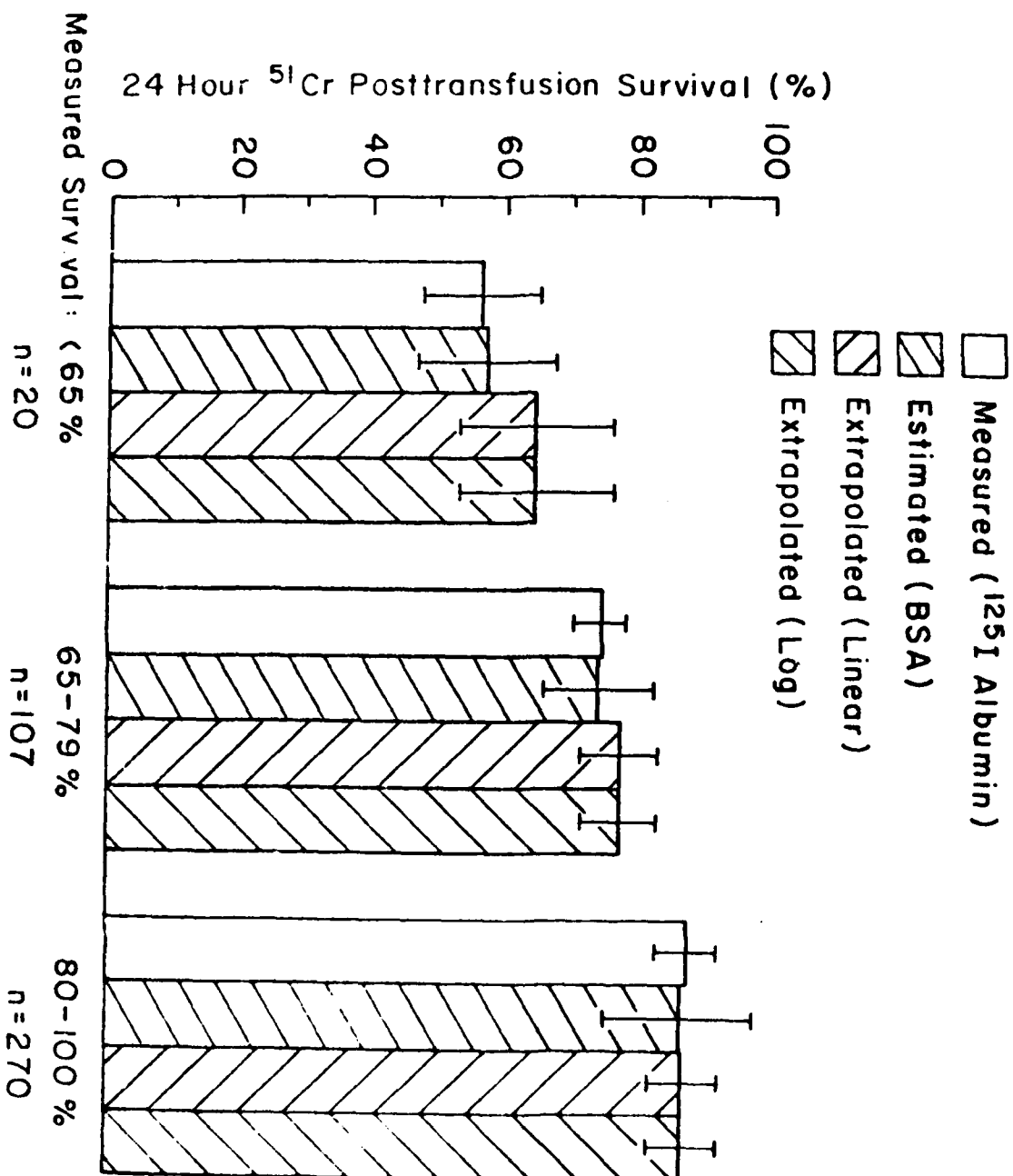
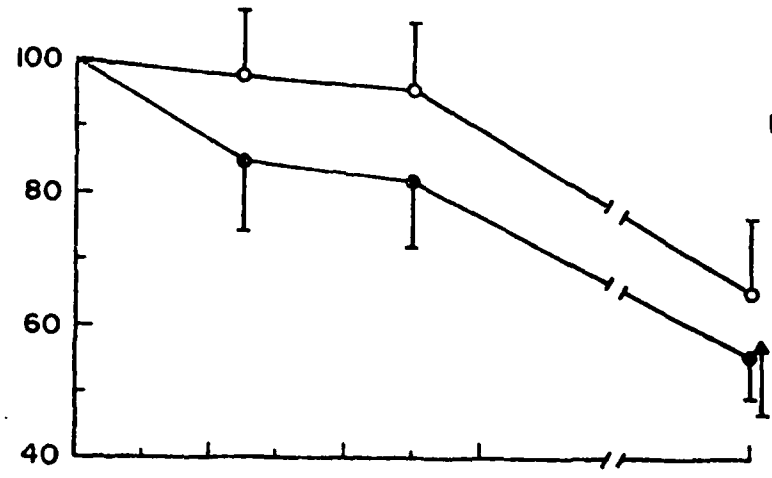


FIGURE 7

The ^{51}Cr survival values of preserved red blood cells during the 24-hour post-infusion period calculated using the measured ^{125}I albumin 100% survival value; the linear and logarithm (log) extrapolated 100% value; and the estimated 100% survival value estimated from the body surface area. The three methods for calculating the ^{51}Cr survival were compared when the 24-hour posttransfusion survival measured using the ^{125}I albumin 100% survival value was less than 65%, 65-79%, and 80-100%.

FIGURE 7
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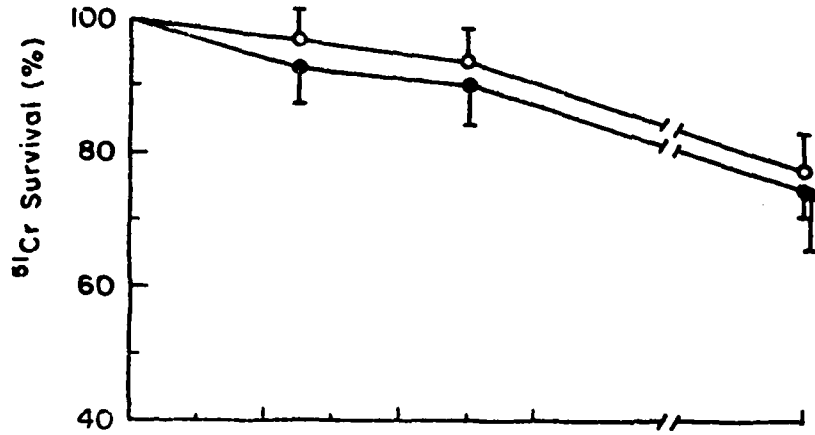
Measured 24 Hour Survival (65% n=20)



Paired t Test at 24 Hr.
 Measured vs. Extrapolated

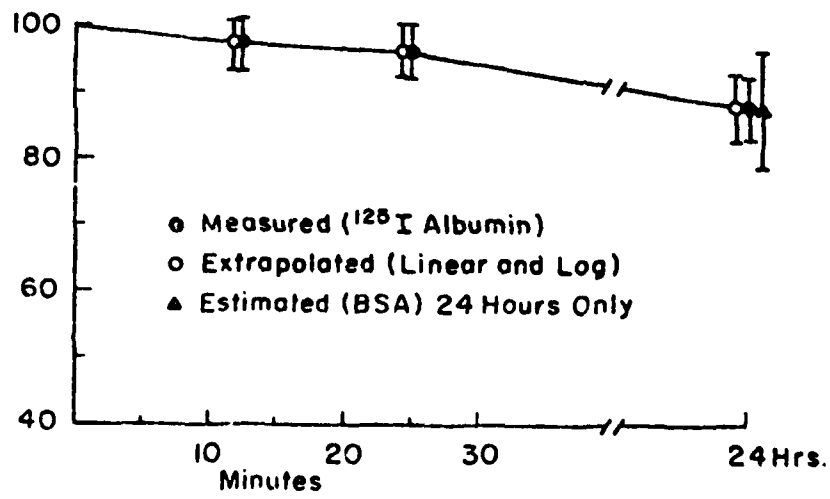
t = 5.34
 p < 0.001

Measured 24 Hour Survival 65-79% n=107



t = 5.97
 p < 0.001

Measured 24 Hour Survival 80-100% n=270



t = 0.08
 p = NS

- Measured (¹²⁵I Albumin)
- Extrapolated (Linear and Log)
- ▲ Estimated (BSA) 24 Hours Only

Time After ⁵¹Cr Labeled Cells Infused

FIGURE 8

^{51}Cr survival of preserved red blood cells during the 24-hour post-transfusion period calculated from the measured ^{125}I albumin 100% survival value, the linear and logarithm extrapolated 100% survival values, and the 100% survival value estimated from the body surface area.

FIGURE 8

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- Measured (^{125}I Albumin)
- Estimated (BSA)
- △ Extrapolated (Linear)
- ▲ Extrapolated (Log)

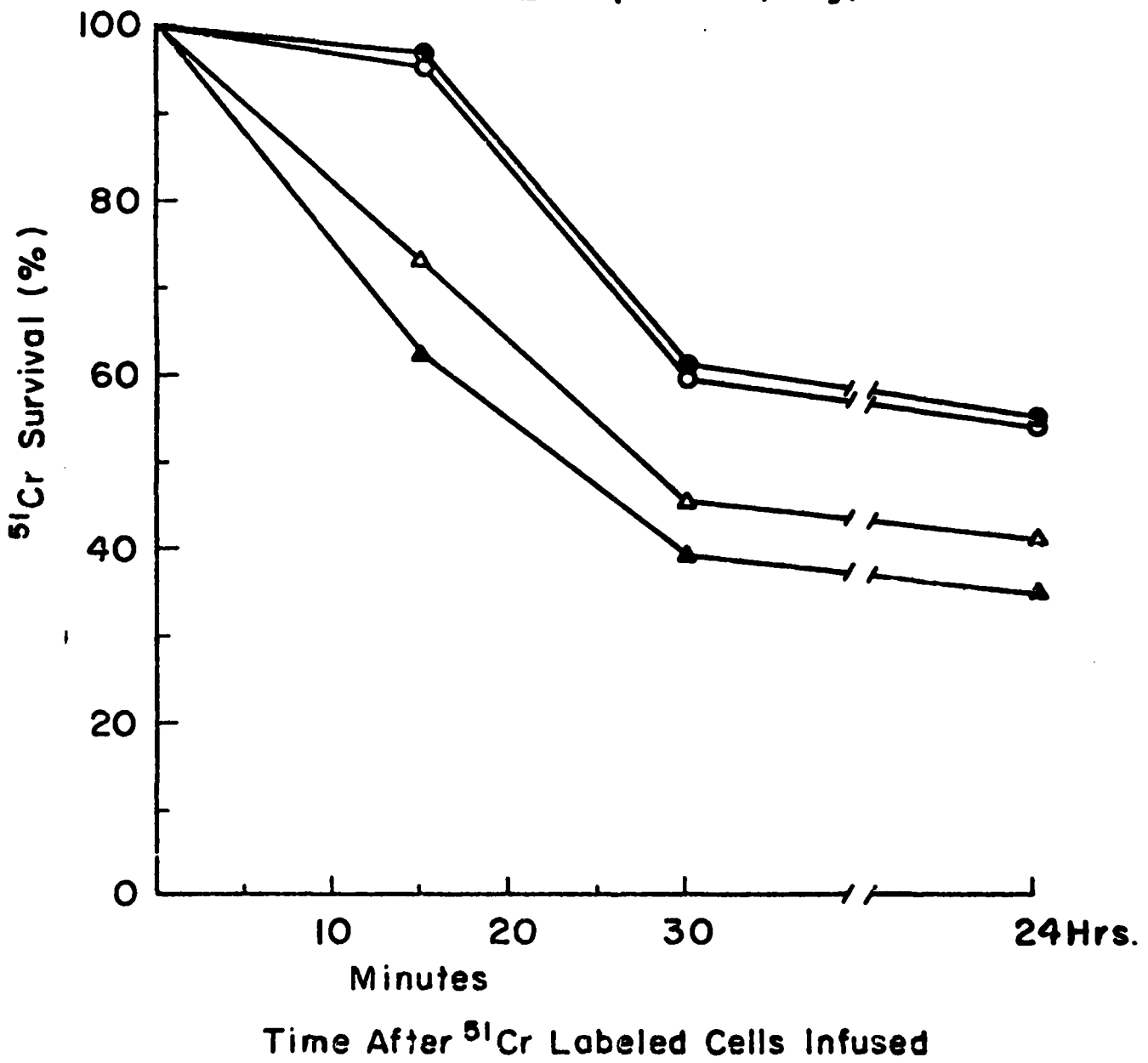


FIGURE 9

In 386 autotransfusions, the frequency distribution in percentage is plotted to show the difference between the 24-hour posttransfusion survival value calculated from the 100% survival value estimated from the body surface area and the 24-hour posttransfusion survival value calculated from the measured ^{125}I albumin 100% survival value.

FIGURE 9
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Difference in ⁵¹Cr 24 Hour Posttransfusion Survival

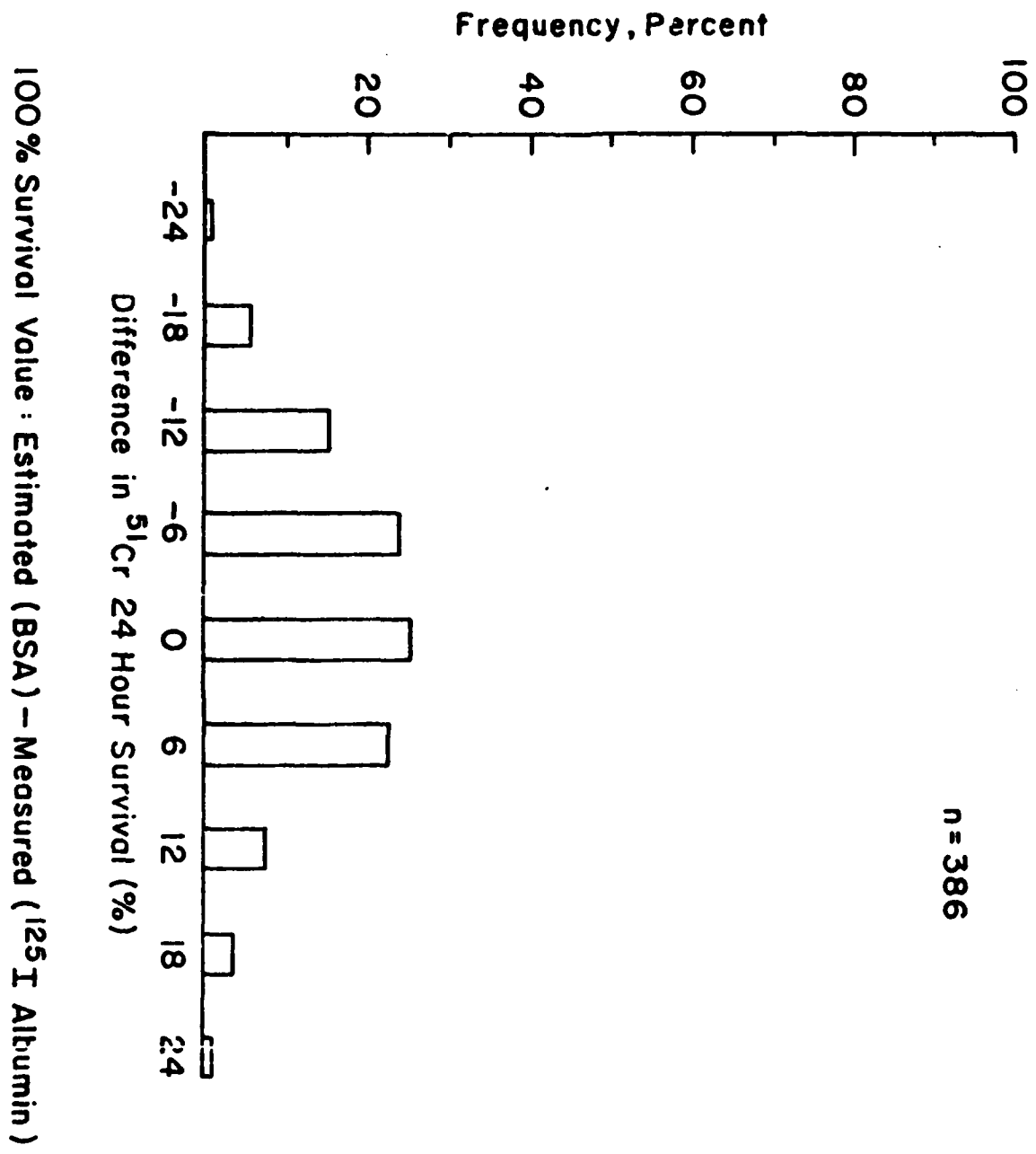
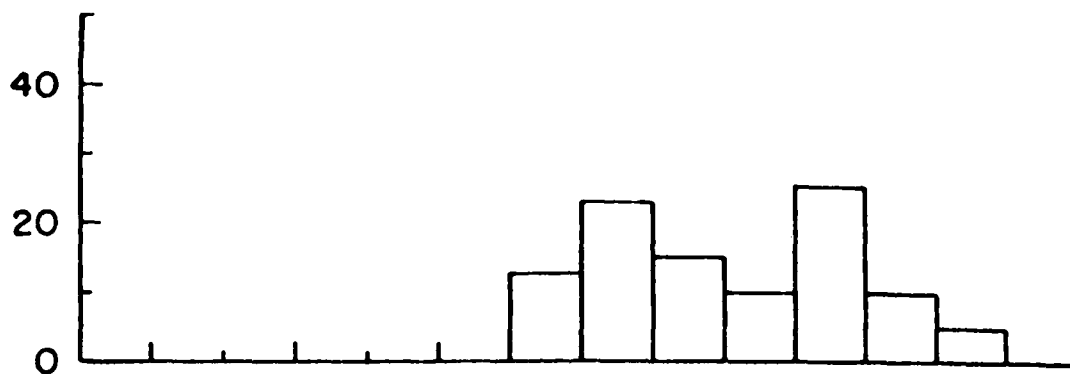


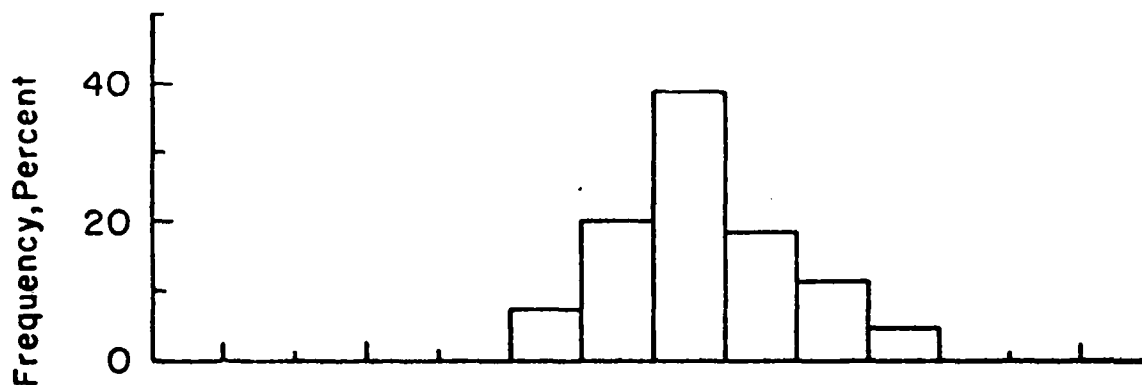
FIGURE 10

The frequency distribution in percentage is plotted to show the difference between the 24-hour posttransfusion survival value calculated from the extrapolated linear 100% survival value and the 24-hour posttransfusion survival value calculated from the measured ^{125}I albumin 100% survival value. The frequency distribution for the difference between the two methods is plotted when the 24-hour ^{51}Cr survival value measured by the ^{125}I albumin 100% survival value was less than 65%, 65-79%, and 80-100%.

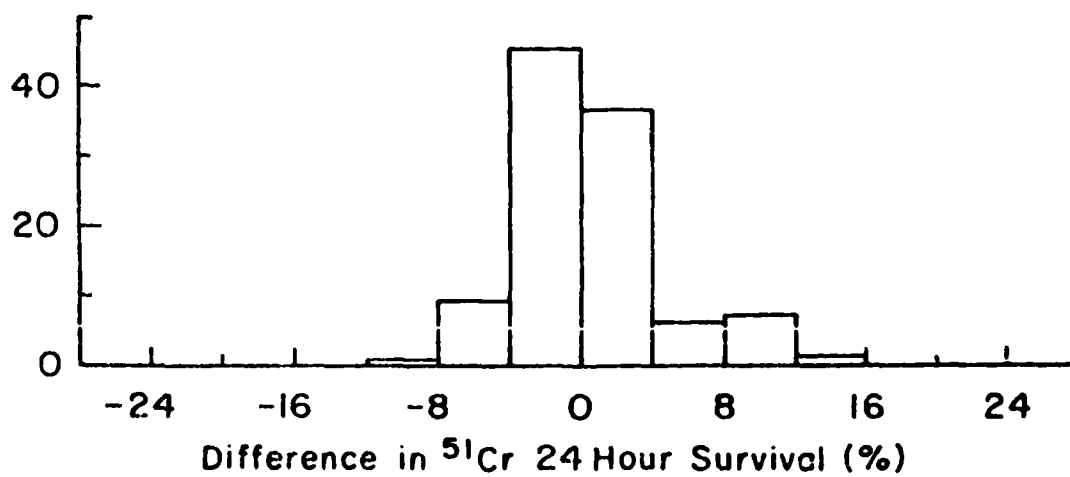
Measured 24 Hour Survival (<65% n=20)



Measured 24 Hour Survival 65-79% n=107



Measured 24 Hour Survival 80-100% n=270



100% Survival : Extrapolated (Linear) - Measured (^{125}I Albumin)

FIGURE 10

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OVERVIEWMEASUREMENT OF THE POSTTRANSFUSION SURVIVAL OF PRESERVED RED BLOOD CELLS

CAPT C. P. VALERI, MC, USN

The injury to red cells produced by preservation in the liquid or frozen state is assessed by labeling the preserved red cells with $^{51}\text{Chromium}$ radioisotope and estimating the dilution of the radiolabeled red cells in the red cell volume of a healthy male normal volunteer.

The $^{51}\text{Chromium}$ -labeled preserved autologous red cells may be lost as the small aliquot is mixed in the red cell volume of the recipient. Red cell volume is measured in a healthy male volunteer by labeling of fresh autologous blood with $^{51}\text{Chromium}$. There is only minimal damage to the red cells during the labeling procedure. Following infusion the $^{51}\text{Chromium}$ -labeled autologous fresh red cells and mixing in the circulation, the red cell volume is estimated from the dilution of the $^{51}\text{Chromium}$ red cells. However, when red cells are stored in the liquid or frozen state before $^{51}\text{Chromium}$ labeling they may have suffered damage and these injured red cells may be lost from the circulation, leading to an overestimation of the recipient's red cell volume when estimated from the dilution of the preserved autologous red cells.

Thus, it is important, when measuring the posttransfusion survival of preserved autologous red cells, that the preserved red cells be labeled with $^{51}\text{Chromium}$ and the recipient's red cell volume estimated independently by measuring the plasma volume of the recipient using iodinated ^{125}I -labeled albumin and the total body hematocrit (peripheral venous hematocrit multiplied by 0.89).

The independent measurement of the red cell volume made indirectly from the measured plasma volume and the total body hematocrit gives a more accurate estimate of the dilution of the $^{51}\text{Chromium}$ -labeled autologous preserved red cells.

Some investigators do not measure the red cell volume of the recipient, but rather obtain several blood samples following the infusion of the small aliquot of chromium-labeled autologous red cells. The radioactivity in counts per minute per ml of red cells is measured during the 30- to 45-minute post-infusion period and an estimate of 100% value is determined by extrapolation of the data to the zero time. The cpm/ml RBC are plotted as linear or log values, and the linear regression of the data obtained during the 30- to 45-minute post-infusion period is extrapolated to zero time. The extrapolation of the data during the immediate post-infusion period to estimate the 100% survival may not detect the red cells lost during the post-infusion period, thereby giving an overestimation of the 24-hour posttransfusion survival by 15-20%, depending upon how the nonviable irreversibly damaged red cells are removed from the circulation during the mixing time.

As an alternative to measuring the red cell volume of the recipient, this value may be estimated in normal healthy volunteers from the body surface area nomogram.

During the past 15 years, more than 500 small aliquot $^{51}\text{Chromium}$ autologous transfusions have been performed at the Naval Blood Research Laboratory, Boston, MA. The data are now being analyzed to compare the 24-hour post-transfusion survival values obtained: (1) from the measured red cell volume of a healthy male volunteer using ^{125}I albumin to measure the plasma volume

and the total body hematocrit; (2) from the red cell volume estimated from the body surface area; and (3) from the 100% survival value estimated from the extrapolation of the radioactivity associated with the red cells during the 30-45 minute posttransfusion period. Data have shown that the immediate loss of the nonviable autologous preserved red cells during the posttransfusion period may not be detected when the 100% survival value is estimated using the extrapolation procedure.

At the Naval Blood Research Laboratory, an aliquot of 20 ml or more of preserved blood is labeled with 0.5 microcuries of disodium ^{51}Cr Chromate per ml of blood and incubated at 37 C for 30 minutes. 1.25 microcuries of ^{125}I albumin are diluted with 12.5 ml of sterile sodium chloride. A 10 ml aliquot of the ^{51}Cr -labeled blood is injected, together with a 5 ml volume of ^{125}I -labeled albumin in sodium chloride. The ^{51}Cr -labeled red cell suspension infused contains 5 microcuries of ^{51}Cr and ^{125}I albumin infused contains 0.5 microcuries of ^{125}I albumin. Blood samples are obtained prior to infusion, at 5, 10 and 20 minutes after infusion, and at 24 hours, 2 or 3 days, and 7 days. In some studies additional samples are collected 14 and 21 days following infusion to measure the T_{50} value. The radioactivity in the whole blood and in the separated plasma collected at 5, 10 and 20 minutes, 24 hours, 2 or 3 days, 7 days, 14 days, and 21 days, is measured and the hematocrit and hemoglobin values in each blood sample are measured. Likewise, the radioactivity in samples of the infused blood and the supernatant of the infused blood is measured. Determinations were made of the levels of radioactivity associated with the red blood cells and plasma. In a normal healthy male volunteer the plasma volume is determined from the total ^{125}I albumin

injected radioactivity and ^{125}I albumin radioactivity in the 10-minute blood sample. The recipient's red cell volume is determined from the ^{125}I albumin plasma volume and the total body hematocrit (peripheral venous hematocrit multiplied by 0.89). The total radioactivity associated with the infused preserved autologous red cells was determined from blood and supernatant radioactivity cpm/ml; the volume of the blood injected and the hematocrit level of the blood injected. The theoretical 100% survival of the ^{51}Cr Chromium-labeled red cells was estimated from the total radioactivity associated with the red cells (cpm) divided by the red cell volume of the healthy volunteer (ml) to calculate the cpm/ml of red cells for the 100% survival value of the labeled red cells. The 24-hour, 3, 7, 14 and 21 day survival values were calculated, and T_{50} values reported. There was no correction in these calculations for elution of the ^{51}Cr label from the red cells following infusion.

PRINCIPLES

PROTOCOL FOR MEASURING THE 24-HOUR POSTTRANSFUSION SURVIVAL OF PRESERVED RED CELLS

Only healthy male volunteers between 18 to 60 years of age are used in these studies. These studies have been approved by the Institutional Review Board of the Boston University School of Medicine. Informed consents for these studies are enclosed (enclosure (1)).

Only autologous transfusions of preserved red cells are performed. Compatibility testing is done at the time of infusion using routine ABO and Rh typing, and a Coombs crossmatch is performed.

The weight and height of the volunteer are recorded, together with all the information required by AABB standards for a routine blood donation.

Donors are interviewed and screened according to AABB standards. The donor is bled one unit, 450 ml, of blood, in the citrate-phosphate-dextrose (CPD), CPDA-1, CPDA-2 or CPDA-3 anticoagulant.

Following the phlebotomy and for a period of at least 3 weeks, the donor's red cell volume is allowed to be restored to normal before the reinfusion of 10 ml of his ⁵¹Chromium-labeled red cell suspension. At the time of reinfusion of a 10 ml aliquot of autologous red cells the donor must be in a steady state, i.e., the rate of red cell production should equal the rate of red cell destruction. Additionally, the blood product must be tested for compatibility and for bacteriologic sterility.

A volume of 20 ml or more of the autologous preserved red cell suspension

is incubated with 0.5 microcuries of disodium ^{51}Cr Chromate per ml of red blood cell suspension at 37 C for 30 minutes. A 10 ml aliquot of the ^{51}Cr Chromium-labeled red cell suspension is injected into the normal healthy volunteer, followed by injection of a 5 ml volume of a solution of 1.25 microcuries of iodinated albumin diluted with 12.5 ml of sterile saline.

Samples of heparinized blood are obtained from the healthy volunteer before and at 5, 10 and 20 minutes, 24 hours, 2 or 3 days, and 7 days after the infusion. Hemoglobin concentration and microhematocrit values are measured in all the samples. In some studies samples are obtained 14 and 21 days following infusion to determine the long-term survival and to calculate the ^{51}Cr T_{50} value.

Aliquots of the ^{51}Cr Chromium-labeled preserved red cell suspension, the supernatant solution of the ^{51}Cr Chromium-labeled red cell suspension, and the iodinated albumin solution are prepared in order to accurately count the radioactivity infused, i.e., the radioactivity associated with the preserved red cells, the radioactivity associated with the supernatant of the ^{51}Cr -labeled preserved red cells, and the radioactivity associated with the iodinated albumin solution. The hemoglobin concentration and the microhematocrit levels in the ^{51}Cr -labeled preserved red cell suspension are measured.

The ^{51}Cr Chromium-labeled preserved red cell suspension is pipetted into 3 aliquots for counting: a 1 ml aliquot of the ^{51}Cr red cell suspension plus 2 ml of water; a 3 ml aliquot of a 1/25 dilution of the ^{51}Cr -labeled red blood cell suspension (1 ml of ^{51}Cr -labeled red cells plus 24 ml of water) and a 3 ml aliquot of a 1/10 dilution of the ^{51}Cr -labeled red cell

suspension (1 ml of ^{51}Cr -labeled red cells plus 9 ml of water). The supernatant of the ^{51}Cr red cell suspension is obtained by centrifugation and a 2 to 3 ml volume of the supernatant of the ^{51}Cr -labeled red cells is pipetted. Three aliquots of the remaining iodinated albumin are pipetted. A 3 ml volume of iodinated albumin solution, a 2 ml volume of iodinated albumin solution plus 1 ml of water, and a 1 ml volume of iodinated albumin solution and 2 ml of water.

Three ml samples of the heparinized blood samples obtained from the healthy male volunteer prior to and following infusion of the radiolabeled ^{51}Cr red cells and the iodinated ^{125}I -labeled albumin solution are pipetted and then the plasma from each sample is obtained by centrifugation and a 2 to 3 ml volume of the plasma from each blood sample is pipetted.

All of the 3 ml volumes of the ^{51}Cr -labeled red cell suspension (1 ml 1/10 and 1/25 dilution of the ^{51}Cr red cell suspension), the 2 or 3 ml volume of the supernatant of the ^{51}Cr -labeled blood, the 3 ml volumes of the iodinated albumin solution (3 ml; 2 ml; and 1 ml) and the 3 ml of the heparinized blood and the 3 ml of the plasma of each heparinized blood sample obtained from the healthy volunteer prior to and following the infusion of the 10 ml radiolabeled ^{51}Cr -labeled red cell suspension and 5 ml of the radiolabeled ^{125}I albumin are counted for radioactivity. All the 3 ml tubes are counted together and there is no need for correction for decay of the radioactivity. Routinely, the blood samples from the volunteer are obtained for 7 days after infusion of the autologous preserved red cells and the iodinated albumin solution. For some studies samples are obtained 14 and 21 days after infusion to determine the T50 value.

With a properly operating and calibrated gamma counter, it is possible to accurately count ^{125}I and ^{51}Cr radioactivity in the blood samples.

The radioactivity in the ^{51}Cr -labeled preserved red cells is determined from the radioactivity in the preserved blood and the supernatant of the preserved blood and from the microhematocrit value. The total radioactivity associated with the ^{125}I -labeled albumin also is determined. The dilution of the ^{125}I -labeled albumin into the plasma volume of the recipient is used to estimate the plasma volume: the 10-minute post-infusion sample is used to calculate the plasma volume. Calculations are made of the plasma volume and the total body hematocrit (peripheral venous hematocrit multiplied by 0.89), the total blood volume, and the red cell volume of the recipient.

The total amount of radioactivity (cpm) in the infused ^{51}Cr -labeled red cells is divided by the red cell volume of the recipient to determine the cpm/ml of red cells and to estimate the 100% survival value. Determinations of the ^{51}Cr radioactivity in the blood and the plasma for each sampling period and the microhematocrit value for each blood sample are used to calculate the cpm/ml of red cells at 5 and 10 minutes, 20 minutes, 24 hours, 3 days and 7 days following infusion. The observed cpm/ml of red cells is compared to the ^{51}Cr radioactivity associated with the injected red cells (cpm) divided by the measured red cell volume (ml of RBC).

Using the weight and height of each volunteer, the body surface area is estimated from the nomogram of Dubois and Dubois (Dubois, D. and Dubois, E. F.: Clinical colorimetry: a formula to estimate the approximate

surface area if height be known. Arch. Intern. Med. 17:863, 1916).

The theoretical red cell volume of males is calculated from the body surface area and the factor 1536. The theoretical plasma volume of males is calculated from the body surface area and the factor 1020. Female volunteers are not used in these studies because of the potential exposure of a fetus to radioisotopes. The total amount of ^{51}Cr radioactivity associated with the preserved red cells (cpm) is divided by the red cell volume of the recipient, calculated from the body surface area to estimate the theoretical 100% survival value in cpm/ml of red cells.

The observed cpm/ml of red cells may be greater than the theoretical cpm/ml of red cells estimated from the measured red cell volume (^{125}I albumin plasma volume and the total body hematocrit) or from the theoretical red cell volume (the body surface area estimate). In this case, the cpm/ml of red cells observed in the 5-, 10- and 20-minute blood samples following infusion of the ^{125}I -labeled albumin and the ^{51}Cr -labeled preserved red cells would be used to estimate the circulating cpm/ml of red cells.

DETAILED PROTOCOLNBRL PROTOCOL FOR MEASUREMENT OF THE POSTTRANSFUSION SURVIVAL OF PRESERVED
RED BLOOD CELLS

1. Adjust hematocrit of preserved blood (normal = 35-55 V%) by adding a sterile sodium chloride solution to the unit.
2. Label an aliquot of the blood (20 ml or more) with 0.5 microcuries per ml of disodium ^{51}Cr Chromate (Chromitope Sodium, Squibb) and incubate at 37 C for 30 minutes in a water bath with gentle mixing.
3. During incubation of the preserved red cells with the ^{51}Cr , the ^{125}I -labeled albumin is prepared as follows:
 - a. Obtain 1 sterile Squibb ACD vial (10 ml of ACD in a 75 ml vial) or a sterile, pyrogen-free glass vial with siliconized interior, large enough to hold at least a 20 ml volume (or 30 ml if you wish to study 2 or more subjects at the same time).
 - b. Remove all ACD from the vial and discard.
 - c. Add 1/2 syringe (approximately 0.75 ml containing approximately 1.25 microcuries of ^{125}I) of radioiodinated ^{125}I human serum albumin (Ames Volemetron RIHSA Dose).
 - d. Add 12.5 ml of 0.9% sodium chloride injection.

NOTE: Alternative: Add 1 full syringe (approximately 1.5 ml containing approximately 2.5 microcuries of ^{125}I) plus 25 ml sodium chloride

- e. Mix well and remove a 5 ml aliquot for injection. Volume must be exact, with no air bubbles. Syringes for ^{51}Cr and ^{125}I aliquots should be calibrated. This is done by filling a syringe and weighing it.
4. During the incubation period, also prepare one syringe containing 10-20 ml of 0.9% sodium chloride injection to be used to flush the intravenous line.
5. After 30 minutes of incubation, mix ^{51}Cr -labeled blood by hand until a homogeneous suspension is obtained.
6. Remove a 10 ml aliquot for injection. Again, volume must be exact, with no air bubbles.
7. As a safety precaution, check the activity of each aliquot using a radioisotope calibrator. Activity should not greatly exceed 5 microcuries for the ^{51}Cr -labeled aliquot and 0.5 microcuries for the ^{125}I -labeled aliquot.
8. Prepare subject by obtaining written consent for the procedure; check pulse, blood pressure, temperature, and hematocrit or hemoglobin. Subject should meet criteria for blood donation as outlined in the American Association of Blood Banks Technical Manual.
9. Insert a 19 g butterfly needle in either arm of subject and secure with surgical tape.
10. Draw "pre-transfusion sample" - 10 ml green top tube (heparinized vacutainer, B-D).

11. Infuse 10 ml of ^{51}Cr -labeled aliquot and flush syringe with blood of the volunteer 2-3 times. Follow this immediately with infusion of 5 ml ^{125}I -labeled aliquot and flush this syringe with blood of the volunteer 2-3 times also. Note time of injection. Flush line with a small amount of 0.9% sodium chloride injection to insure that all isotope enters the bloodstream (use syringe prepared earlier to flush the intravenous line).

NOTE: It is crucial that the entire contents of the aliquot syringes are infused and flushed since in the calculations you will assume that the exact volume (10 ml for the ^{51}Cr /5 ml for the ^{125}I) entered the bloodstream; any infiltration, therefore, will invalidate results.

12. Attach a 0.9% sodium chloride slow drip to the butterfly. This will prevent clotting of the line between sampling times.
13. Draw followup samples at 5, 10 and 20 minutes post-infusion of ^{125}I aliquot (10 ml green top for each).

NOTE: Line must be cleared of saline before drawing actual samples. Additional followup samples are drawn at 24 hours, 48 or 72 hours, 7, 14 and 21 days post-infusion (10 ml green top for each). All samples should be placed on an aliquot mixer until needed.

PROCESSING OF SAMPLES

14. Mix remaining ^{51}Cr -labeled blood by hand until a homogeneous suspension is obtained and remove a 10 ml aliquot for pipetting into a red top tube. Place on aliquot mixer until needed.
15. Remove a 7 ml aliquot for pipetting from ^{125}I vial into a red top tube and place on mixer.
16. Perform hematocrit on all blood samples: pre-transfusion, 5-, 10- and 20-minute post-transfusion, ^{51}Cr injectate on the day of the study.
NOTE: Accurate measurement of hematocrit is essential to calculation of blood volume and survival. Therefore, use microhematocrit tube method and take the average of two readings.
17. Pipette 3 ml of whole blood (well mixed) from each blood sample, except ^{51}Cr injectate, into gamma counting tubes. Use Class A "unserialized" volumetric T.D. (to deliver) pipettes.
18. Pipette dilutions of the ^{51}Cr injectate (well mixed) into 3 gamma tubes as follows: (a) 1 ml of non-diluted; (b) 3 ml of a 1:10 dilution; (c) 3 ml of a 1:25 dilution.
Use volumetric flasks for dilutions to ensure accuracy; dilute blood with water so that cells will hemolyze.
19. QS all gamma tubes to 3 ml with water; cap and label tubes.
20. For all samples spin remaining blood in a clinical centrifuge for 10 minutes at approximately 3000 rpm to separate plasma or supernatant from the red cells.

21. Carefully pipette off plasma or supernatant into another gamma tube (or other test tube) using pasteur pipettes. Care must be taken to avoid red cells in the supernatant.
22. Pipette 2 or 3 ml of the plasma or supernatant into another gamma tube using volumetric pipettes; QS to 3 ml with water; cap and label tubes.
23. Pipette dilutions of the ^{125}I injectate into 3 gamma tubes as follows:
(a) 1 ml; (b) 2 ml; (c) 3 ml.
QS all tubes to 3 ml with water; cap and label tubes.
24. Culture the remaining ^{51}Cr injectate and check daily for growth for 7 days; culturing ^{125}I injectate is optional; remainder of both injectates should be saved for at least 7 days following the trans- fusion in case future investigation is necessary.

GAMMA COUNTING OF SAMPLES (USUALLY PERFORMED ONCE AFTER 24-HOUR FOLLOW-UP AND AGAIN AFTER 7-DAY FOLLOWUP) AND CALCULATIONS:

25. Count all gamma tubes as a group (in this manner, no correction for radioactive decay need be made) and include a tube to measure back-ground (BKG) radiation.
NOTE: BKG tube consists of an empty gamma tube.
26. Count ^{51}Cr and ^{125}I simultaneously - 5 minute counts X 3.
27. Calculate average counts per minute (AVG CPM) of both ^{51}Cr and ^{125}I for each sample.

28. Normalize AVG CPM for ^{51}Cr and ^{125}I dilutions to 1 ml in the following manner:

For ^{51}Cr :	<u>Dilution</u>	<u>to Normalize</u>	
	1 WB	X 1 (no change)	
	3 1:10	X 10, \div 3	then calculate AVG CPM/ml
	3 1:25	X 25, \div 3	
For ^{125}I :	<u>Dilution</u>	<u>to Normalize</u>	
	3 ml	\div 3	
	2 ml	\div 2	then calculate AVG CPM/ml
	1 ml	\div 1	

Radiation Safety Note: Work with radioisotopes should be performed only in areas designated as radiation work areas. When working with radioisotopes always wear a film badge to monitor your exposure, and allow yourself as little exposure as possible. Always wash your hands immediately after working with isotopes. Dispose of all contaminated materials properly in the appropriate radioactive waste barrels. All containers (bags, vials, syringes, tubes, etc) with radioactive material should have appropriate warning labels affixed.

Blood Volume Measurement Note: Beginning in January 1982, radioiodinated human serum albumin (RIHSA) will no longer be available from Ames. A similar product is available, however, in a more concentrated form (i.e., more activity per ml) from Mallinckrodt. Use of this product would require only a slight change in protocol. The final concentration of the injectate should remain 0.1 microcuries per ml. Please note that this manufacturer's product has not yet been evaluated at NBRL.

Formula 1.

$$\text{PLASMA VOLUME (ml)} = \frac{(\text{AVG CPM/ml of } ^{125}\text{I injectate} - \text{BKG})(\# \text{ ml's injected})}{\left(\frac{\text{AVG CPM in f/u plasma} - \text{BKG}}{\# \text{ ml's plasma pipetted}} \right) - \left(\frac{\text{AVG CPM in PRE plasma} - \text{BKG}}{\# \text{ ml's plasma pipetted}} \right)}$$

$$\text{RED CELL VOLUME (ml)} = \frac{(\text{AVG HCT.})(\text{"f" factor})}{1 - (\text{AVG HCT.})(\text{"f" factor})} (\text{plasma volume (ml)})$$

where "f" factor = 0.89

NOTE: Plasma volume is calculated for the 10-minute and 20-minute follow-ups only. The red cell volume is calculated for the 5-, 10- and 20-minute followups. "AVG HCT" in the formula for red cell volume refers to an average of the PRE and 5- and 10-minute hematocrits for the 10-minute red cell volume and an average of the PRE, 5-, 10- and 20-minute hematocrits for the 20-minute red cell volume; hematocrit in this and in all formulas is expressed as a decimal.

NOTE: "HCT" in the formula for % survival refers to the hematocrit of the individual sample (either of the PRE, f/u, or injectate sample, as required).

The term enclosed by brackets [] in the % survival formula, when multiplied by 100, gives the % ^{51}Cr uptake (i.e., the % of the ^{51}Cr activity that is red cell-associated); if you do not wish to calculate % uptake, then some cancellation of terms is possible. % ^{51}Cr uptake for washed red cells is typically 90-98%; for whole blood the uptake may be 5-10% lower.

When the % survival at 10 or 20 minutes is greater than 100% (usually due to an overestimation of red cell volume), use the "% circulating survival" formula. In this formula you assume that the first f/u obtained = 100% and then relate subsequent f/u samples to the first.

When AVG CPM in PRE whole blood is \leq , AVG CPM in BKG, the entire PRE term becomes zero.

STATEMENT OF INFORMED CONSENTPROCUREMENT OF A UNIT OF BLOOD

SUMMARY: You are being asked to donate a unit (1 pint) of your blood for research investigations of methods to improve the liquid and freeze-preservation of red blood cells, white blood cells, platelets and plasma proteins.

PROCEDURE: Approximately 1 pint of your blood will be collected from one of your arm veins into a blood bag made of a standard or new plastic material which contains a standard or new anticoagulant.

POTENTIAL RISKS AND HAZARDS: Possible risks and discomforts are the pain of inserting the needle in your arm to draw the blood, fainting, infection, and a bruise at the site of needle insertion.

BENEFITS: Monetary considerations and personal satisfaction aside, you will derive no health or other benefits from your participation in this study.

VOLUNTEER SUBJECT CONSENT

I have read and understand the above explanation and the risks of participating in this blood donation. Details of this procedure and potential risks and hazards have been verbally explained to me and I have had a chance to ask questions which have been answered to my satisfaction. I understand that any new information developed during the course of the research that may relate to my willingness to continue participation will be provided to me. I understand that I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled. I further understand that my identity will be known only to the investigators and I will not be identified by name in any publication resulting from this study. However, the Food and Drug Administration may inspect my record. I authorize the attending physician, his assistants or designees to perform such therapies or procedures which in his professional judgment may become necessary to safeguard my health during the study.

I understand that in the event physical injury occurs resulting from this research procedure, medical treatment will be available at University Hospital. However, no special arrangements will be made for compensation or for payment for treatment solely because of my participation in this experiment. I understand that this paragraph is a statement of University Hospital's policy and does not waive any of my legal rights.

If there are any questions or problems, I am to call the Laboratory at 247-6700 during normal work hours and 247-5042 nights and weekends.

Signature of Witness

Signature of Volunteer

Signature of Investigator

Date

STATEMENT OF INFORMED CONSENTDONATION OF UP TO 100 ML OF WHOLE BLOOD

SUMMARY: You are being asked to donate a blood sample to collect information which could be used to improve the supply and usefulness of red cell, platelet, and white blood cell transfusions.

PROCEDURE: Up to 100 ml (1/5 pint) of your blood will be collected by inserting a needle in your arm vein.

POTENTIAL RISKS AND HAZARDS: There are no known risks involved with the procedure, except for a possible bruise at the site of needle insertion.

BENEFITS: Monetary considerations and personal satisfaction aside, you will derive no health or other benefits from donating your blood.

VOLUNTEER SUBJECT CONSENT

I have read and understand the above explanation and the risks of participating in this blood donation. Details of this procedure and potential risks and hazards have been verbally explained to me and I have had a chance to ask questions which have been answered to my satisfaction. I understand that I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled. I further understand that my identity will be known only to the investigators and I will not be identified by name in any publication resulting from this study. However, the Food and Drug Administration may inspect my record. I authorize the attending physician, his assistants or designees, to perform such therapies or procedures which in his professional judgment may become necessary to safeguard my health during this blood donation.

I understand that in the event physical injury occurs resulting from this blood donation, medical treatment will be available at University Hospital. However, no special arrangements will be made for compensation or for payment for treatment solely because of my participation in this blood donation. I understand that this paragraph is a statement of University Hospital's policy and does not waive any of my legal rights.

If there are any questions or problems, I am to call the Laboratory at 247-6700 during normal work hours and 247-6042 nights and weekends.

Signature of Witness

Signature of Volunteer

Signature of Investigator

Date

STATEMENT OF INFORMED CONSENTSURVIVAL AND FUNCTION OF BIOCHEMICALLY MODIFIED, FREEZE-PRESERVEDHUMAN RED BLOOD CELLS

SUMMARY: You are being asked to participate in a research study involving the collection of a unit of your blood (about 1 pint), storing it in a refrigerator for up to 7 weeks, then treating it with a chemical solution, freezing and thawing it, and injecting a small portion of it (about 2 teaspoons) into your veins after adding small amounts of radioactive isotopes to measure your red cell survival. We want to see if a procedure that we have developed for restoring depleted elements in red blood cells will be affected by freeze-preservation and storage in a refrigerator for up to 7 days after thawing and washing.

PROCEDURE: We will collect a unit of your blood into a blood bag containing a standard anticoagulant (ACD, CPD, CPD-A₁). The blood will be stored as whole blood or packed cells in a standard blood bank refrigerator for up to 7 weeks. The red blood cells will be mixed with a special solution which restores certain elements that were lost from the red cells during storage and are necessary for the cells to function properly after transfusion. Following this rejuvenation procedure the cells will be frozen and stored in a freezer for a few days. After thawing and washing, the cells will again be stored in a refrigerator for up to 7 days. In order to determine how many of these cells will tolerate this procedure and survive in the circulation after transfusion, a small amount of radioactive ⁵¹chromium will be added to a sample of the cells. About 2 teaspoons of this sample and a small amount of radioactive iodine will be injected into your veins. You will receive 0.5 microcuries of radioactive ¹²⁵Iodine labeled albumin and 5 microcuries of radioactive ⁵¹Cr disodium chromate. Blood samples will be collected prior to the injection and at 10 minutes, 20 minutes, 24 hours, 2 or 3 days and 7 days after the injection. The total amount of blood collected will be about 3 ounces.

POTENTIAL RISKS AND HAZARDS: There is a remote chance that you might mistakenly be injected with another donor's blood and that this might possibly cause a reaction or disease (especially hepatitis), but a strict identification system virtually excludes this possibility. Another possibility is that your blood might become contaminated during the procedure, but your blood will be checked for this before it is injected back into your veins. There is also a slight risk that the radioactive iodine might cause a reaction when injected. There might be an unknown risk associated with adenine in the anticoagulant. There are no risks known to be associated with the amount of radioactivity that you will be exposed to in this study. The thyroid, the single organ subjected to the largest amount of ¹²⁵Iodine radiation during this study, receives less than 1/5 of the permissible exposure for a single study as outlined by the Food and Drug Administration. Total body radiation from ⁵¹Cr and from ¹²⁵Iodine together is less than 1/250 of the permissible exposure for a single study.

BENEFITS: Monetary considerations and personal satisfaction aside, you will derive no health or other benefits from your participation in this study.

VOLUNTEER SUBJECT CONSENT

I have read and understand the above explanation and the risks of participating in this study. Details of this procedure and potential risks and hazards have been verbally explained to me and I have had a chance to ask questions which have been answered to my satisfaction. I understand that any new information developed during the course of the research that may relate to my willingness to continue participation will be provided to me. I understand that my blood will be injected back to me after it has been manipulated in the laboratory and that I am asked to provide blood samples for a few days after the injection. However, I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled. I further understand that my identity will be known only to the investigators and I will not be identified by name in any publication resulting from this study. However, the Food and Drug Administration may inspect my record. I authorize the attending physician, his assistants or designees to perform such therapies or procedures which in his professional judgment may become necessary to safeguard my health during this study.

I understand that in the event physical injury occurs resulting from this research procedure, medical treatment will be available at University Hospital. However, no special arrangements will be made for compensation or for payment for treatment solely because of my participation in this experiment. I understand that this paragraph is a statement of University Hospital's policy and does not waive any of my legal rights.

If there are any questions or problems, I am to call the Laboratory at 247-6700 during normal work hours and 247-6042 nights and weekends.

Signature of Witness

Signature of Volunteer

Signature of Investigator

Date

STATEMENT OF INFORMED CONSENTSURVIVAL AND FUNCTION OF HUMAN RED BLOOD CELLS FREEZE-PRESERVED AND PERFUSED
THROUGH AN EXTRACORPOREAL CIRCUIT PRIOR TO AUTOTRANSFUSION

SUMMARY: You are being asked to participate in a research study involving the collection of a unit of your blood (about 1 pint), storing it in a standard refrigerator at 4 C for up to 7 weeks, then treating it with a chemical solution, freezing and thawing it, storing it again in a standard refrigerator for up to 3 days, and then passing the red cells diluted with a solution through an artificial lung for up to 3 hours, and injecting a small portion of it (about 2 teaspoons) into your veins after adding a small amount of radioactive solution. We want to see if a procedure that we have developed for maintaining or improving the red blood cell's ability to carry and deliver oxygen will be affected by passing the red cells through an artificial lung.

PROCEDURE: We will collect a unit of your blood into a blood bag containing a standard anticoagulant (ACD, CPD, CPD-A₁). The blood will be stored in a standard blood bank refrigerator as whole blood or packed cells for up to 7 weeks. The red blood cells will then be mixed with a special solution which restores certain elements that were lost from the red cells during storage and are necessary for the cells to function properly after transfusion. Following this rejuvenation procedure the cells will be frozen in the routine manner and stored in a freezer for a few days. After thawing and washing, the cells will again be stored in a refrigerator for up to 3 days. The red cells will be passed through an artificial lung for up to 3 hours. In order to determine how many of these cells will tolerate passage through an artificial lung that is routinely used during surgical procedures on the heart and survive in the circulation after transfusion, a small amount of radioactive chromium will be added to a sample of the cells. About 2 teaspoons of this sample and a small amount of radioactive iodine will be injected into your veins. You will receive 0.5 microcuries of radioactive ¹²⁵Iodine labeled albumin and 5 microcuries of radioactive ⁵¹Cr disodium chromate. Blood samples will be collected prior to the injection and at 10 minutes, 20 minutes, 24 hours, 2 or 3 days and 7 days after the injection. The total amount of blood collected will be about 3 ounces.

The procedure described above is performed routinely in our laboratory. The only thing that is different about this study is that the preserved red blood cells will be passed through an artificial lung before reinfusion.

POTENTIAL RISKS AND HAZARDS: There is a remote possibility that you might mistakenly be injected with another donor's blood and that this might possibly cause a reaction or disease (especially hepatitis), but a strict identification system virtually excludes this possibility. Another possibility is that your blood might become contaminated during the procedure, but your blood will be checked for this before it is given back to you. There might be unknown risks associated with adenine in the anticoagulant. There is also a slight risk that the radioactive iodine might cause a reaction when injected. There are no risks

known to be associated with the amount of radioactivity that you will be exposed to in this study. The thyroid, the single organ subjected to the largest amount of ^{125}I Iodine radiation during this study, receives less than 1/5 of the permissible exposure for a single study as outlined by the Food and Drug Administration. Total body radiation from ^{51}Cr and from ^{125}I Iodine together is less than 1/250 of the permissible exposure for a single study.

BENEFITS: Monetary considerations and personal satisfaction aside, you will derive no health or other benefits from your participation in this study.

VOLUNTEER SUBJECT CONSENT

I have read and understand the above explanation and the risks of participating in this study. Details of this procedure and potential risks and hazards have been verbally explained to me and I have had a chance to ask questions which have been answered to my satisfaction. I understand that any new information developed during the course of the research that may relate to my willingness to continue participation will be provided to me. I understand that my blood will be injected back to me after it has been manipulated in the laboratory and that I am asked to provide blood samples for a few days after the injection. However, I may discontinue participation at any time without penalty or loss of benefits to which I am otherwise entitled. I further understand that my identity will be known only to the investigators and I will not be identified by name in any publication resulting from this study. However, the Food and Drug Administration may inspect my record. I authorize the attending physician, his assistants or designees to perform such therapies or procedures which in his professional judgment may become necessary to safeguard my health during this study.

I understand that in the event physical injury occurs resulting from this research procedure, medical treatment will be available at University Hospital. However, no special arrangements will be made for compensation or for payment for treatment solely because of my participation in this experiment. I understand that this paragraph is a statement of University Hospital's policy and does not waive any of my legal rights.

If there are any questions or problems, I am to call the Laboratory at 247-6700 during normal work hours and 247-6042 nights and weekends.

Signature of Witness

Signature of Volunteer

Signature of Investigator

Date