# Reference Values for Red Cell Survival Times

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The first purpose of this investigation was to investigate in 35 young normal male subjects the use of the Dornhorst function and the weighted-mean method to calculate reference values for mean red cell survival time with and without correction for elution of $^{51}Cr$. We compared survival times calculated with the Dornhorst and weighted-mean methods with survival time estimated with linear or exponential models. Two methods to correct for elution of $^{51}Cr$ from red cells were investigated. For the first method, correction factors were generated using the Dornhorst function fitted to mean survival curves obtained from the normal subjects. In the second method, the new Dornhorst rate constant method, the survival time, corrected for elution of $^{51}Cr$, was directly calculated from the experimental survival curve without applying correction factors. Correction for elution using the Dornhorst rate constant method was not successful and resulted in nonphysiologic values. The 95% confidence range of red cell survival time for reference subjects without correction for $^{51}Cr$ elution was 37–74 days for the weighted-mean method and 37 to 73 days for the Dornhorst method. The 95% confidence range for normal subjects when the survival curves were corrected for elution was 47–179 days for the Dornhorst method and 58–161 days for the weighted-mean method. The poor results obtained with the Dornhorst rate constant method and the large 95% confidence range were due to the rapid and large variation in elution rate of $^{51}Cr$ from red cells.

**J Nucl Med 1991; 32:2245–2248**

Red cell survival studies using $^{51}Cr$ labeling have been valuable in assessing the nature and severity of hemolytic anemia and the compatibility of transfused blood. Red cells have a finite life span and when it is reduced, as may happen in various pathologic conditions, hemolytic anemia develops. Hemolytic anemia can be divided into two large groups on a pathophysiologic basis: in the first, the red cell life span is shortened due to fundamental defects of the red cell (intracorpuscular defects), e.g., hereditary spherocytosis and paroxysmal nocturnal hemoglobinuria. In the second group, normal red cells are produced, but their life span is reduced due to extracorpuscular mechanisms such as red cell antibodies. In the second group, red cells are randomly destructed, irrespective of age, and at a constant rate.

Dornhorst proposed a general function for red cell survival in normal subjects as well as patients with hemolytic anemia (*1*):

$$D_R = D[\exp(-k_R t) - \exp(-k_R T)]/[1 - \exp(-k_R T)],$$

where T = potential survival time, $k_R$ = rate of random destruction, and D = y-intercept of the Dornhorst function.

In normal subjects, the random rate of red cell destruction ($k_R$) is small and a near linear survival curve will be obtained. The survival time will be slightly shorter than the potential survival time (T). In patients with intracorpuscular red cell defects, the survival curve will also be linear, but with an abnormal senescence mechanism, resulting in a shorter potential survival time (T). With extracorpuscular red cell defects, e.g., autoimmune hemolytic anemia, $k_R$ is large, resulting in an exponential survival curve.

Although the Dornhorst theory has been accepted by several investigators (*2–10*), the Dornhorst function has only been used to a limited extent (i.e., the linear and exponential functions, for the calculation of red cell survival). This is in accordance with the recommendations of the International Committee for Standardization in Hematology (ICSH) if computing facilities are not available (*11,12*). This method (ICSH method) requires the fitting of a linear and exponential function to the survival curve. The survival time obtained with the function that fits the survival curve best is selected. This method has a limitation when the shape of the survival curve is between that of the linear and exponential function and a poor fit and inaccurate survival times are obtained with both functions. However, this disadvantage can be overcome by the use of the weighted-mean method (*6*).

The ICSH proposes that the Dornhorst function should be used for calculation of the red cell survival time if computing facilities are available. However, limited information is available on the value of this method for red cell survival studies, as it has not been directly used to calculate red cell survival time, or to generate elution correction factors, or to correct for elution of the $^{51}Cr$ label. These parameters have been fully investigated in platelet survival studies (*5*).

Received Jan. 30, 1991; revision accepted Jun. 26, 1991.

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**TABLE 1**
Comparison of Published Measured Elution Rates of $^{51}Cr$ from Red Cells

| Author | AVG %/day | s.d. %/day | %s.d. %(%/day) |
|---|---|---|---|
| Cline and Berlin (*9*) | 1.25 | 0.46 | 37 |
| Garby and Mollison (*10*) | 1.20 | 0.43 | 36 |
| Bentley et al. (*8*) | 1.01 | 0.26 | 25 |

Chromium-51 in the form of sodium chromate is the most commonly used radionuclide for random labeling of red cells. However, the variable elution rate of $^{51}Cr$ from red cells contributes to the inaccuracy of survival time estimate. Bentley et al. (*8*) reported an elution rate of 1.01% ± 0.26% in 13 normal subjects (Table 1). In patients, elution rates of 1.25% ± 0.43% per day were reported by Cline and Berlin (*9*) and of 1.2% ± 0.43% per day by Garby and Mollison (*10*). Correction factors have been proposed by Garby and Mollison as well as by Bentley et al. The correction factors by Garby and Mollison have been adopted by the ICSH (*11,12*). We used an equation based on the Dornhorst function for the calculation of correction factors at any stage during red cell survival.

The Dornhorst theory assumes that red cells will be removed from the circulation when they reach the end of their life span, T. It also assumes that some red cells are randomly destroyed, irrespective of its age at rate $k_R$. It is not possible to distinguish between the removal of radioactivity from the circulation by elution or by random destruction of labeled cells. Therefore if the rate of elution ($k_E$) is predetermined, and the rate of removal of radioactivity ($k_T$) is determined using the Dornhorst function, the rate of random red cell destruction ($k_R$) can be calculated:

$$k_R = k_T - k_E.$$

The elution-corrected survival time can then be calculated from random rate of destruction, $k_R$, and the potential survival time, T. This method is known as the Dornhorst rate constant method.

The Dornhorst function requires sophisticated non-linear curve fitting methods that are now available on IBM personal computers. We used the program, "Calculation of Survival Time" (COST), in this investigation (*7*). The first aim of this study was to investigate the use of the Dornhorst function as well as the weighted-mean method to calculate reference values for mean red cell survival time, with and without correction for elution of $^{51}Cr$ in 35 young males using the ACD method proposed by the ICSH (*11,12*). The Dornhorst rate constant method mentioned above was also used to calculate red cell survival time corrected for elution of $^{51}Cr$. The second aim of the study was to investigate elution correction by comparing the correction factor and the Dornhorst rate constant method.

## METHODS

Red cell survival studies were performed in 35 consenting normal male subjects. These subjects were in a clinical trial to determine blood loss after administration of anti-inflammatory medication. Mean blood loss measured by counting stool samples was 28 ml over a period of 44 days and it did not influence red cell survival.

Red cells were labeled using the method described by the ICSH (*11,12*). Briefly, blood was obtained by venipuncture and mixed with ACD. Packed cells were obtained by centrifugation after which sodium chromate ($^{51}Cr$) was added. After incubation, the red cells were washed twice with isotonic saline and then reinjected. Blood samples were drawn on Days 1, 2, 3, 14, 15, 16, 28, 29, 30, 42, 43 and 44. Blood samples were counted in a scintillation counter and the acquired counts were corrected for decay and hematocrit.

Survival curves were fitted using the linear, exponential, weighted-mean and Dornhorst functions and the computer program COST. Survival time was first calculated without correction for elution.

The ICSH did not publish correction factors based on the Dornhorst function. New correction factors were calculated. This was done by first fitting the Dornhorst function to the average survival curve for all the patients with the potential survival time fixed at T = 113 days. Correction factors (CF) to transform the survival curve to an ideal normal linear survival line with a survival time of 113 days were then calculated by the equation:

$$CF = \frac{[1 - t/T]}{D[\exp(-k_E t) - \exp(-k_E T)]/[1 - \exp(-k_E T)]},$$

where T = 113 days, $k_E = 0.0155\ day^{-1}$, and D = 0.973 and is the y-intercept calculated during curve fitting.

The correction factors were applied and red cell survival time was redetermined.

Red cell survival time (RCST), corrected for elution with the Dornhorst rate constant method, was calculated by:

$$RCST = \frac{[1 - \exp(-k_R T)]}{k_R},$$

where $k_R = k_T - k_E$.

The value of the rate of removal of radioactivity $k_T$ and potential survival time, T, were determined when the Dornhorst function was fitted to the uncorrected survival curve data. The value of the rate of elution $k_E$ was taken as the average values obtained from the reference subjects. It was assumed that in these subjects random destruction was not present.

## RESULTS

### Comparison of Mathematical Functions Used for Curve Fitting

In Figure 1, the mean red cell survival time calculated by the ICSH and the weighted-mean methods are correlated. There is perfect agreement for some of the survival times obtained from pure linear or exponential-shaped survival curves. Poor agreement resulted for those studies when the shape of the experimental survival curve was between the linear and exponential regression curves. Figure 2 indicates a good correlation (r = 0.99) between the

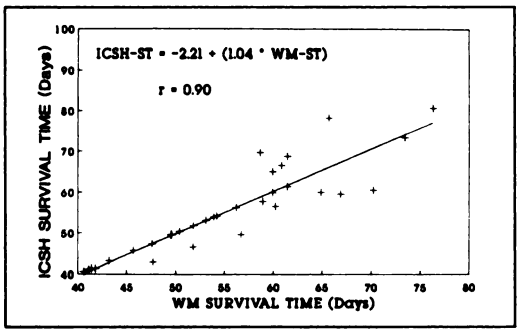


**FIGURE 1.** The correlation between the survival times obtained with the ICSH and weighted mean functions is demonstrated. Poorer agreement was obtained in some patients when the shape of the survival curve was between linear and exponential regression curves.

survival times obtained with the weighted-mean and Dornhorst functions. The results indicate that the weighted-mean or Dornhorst functions are more reliable than the ICSH method for calculating mean red cell survival time. The weighted-mean method is the method of choice to calculate red cell survival time if computing facilities are not available. The weighted-mean method can be calculated with a hand-held scientific calculator.

### Reference Range for Red Cell Survival Times Without Correction for $^{51}$Cr Elution

Reference values of the survival times presented in Table 2 are in agreement with the corresponding results calculated using the survival curve published by the ICSH. Survival time was 55.4 ± 9.0 days with the weighted-mean method and 54.9 ± 8.8 with the Dornhorst function. The 95% confidence reference range for survival times not corrected for elution was 37–74 days for the weighted-mean method and 37 to 73 days for the Dornhorst method (Table 2). The estimated red cell survival times for the weighted-mean method and the Dornhorst function were not different.

### Correction for Elution

Figure 3 demonstrates that if correction for $^{51}$Cr elution is not applied, the mean survival curve (curve 2) measured

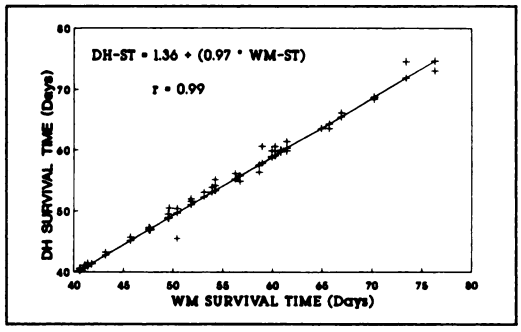


**FIGURE 2.** Good correlation was obtained between the survival times obtained with the Dornhorst function and the weighted mean function.

**TABLE 2**
Mean Red Cell Survival Time in Days Calculated for Different Functions

| Method | Reference subjects | ICSH curve |
|---|---|---|
| Linear | 76.6 ± 6.7 | 62.5 |
| Exponential | 50.6 ± 6.6 | 47.0 |
| ICSH | 55.5 ± 10.4 | 62.5 |
| Weighted-Mean | 55.4 ± 9.0 | 55.0 |
| Dornhorst | 54.9 ± 8.8 | 54.1 |

in this study closely corresponds with the ICSH published survival curve (curve 1). Figure 3 also demonstrates that there is a small difference over a period of the initial 30 days between the mean survival curve corrected for elution in this study (curve 5) and the ICSH survival curve corrected for elution (curve 4). This difference will increase for longer periods and results in a large difference for potential survival times. Good agreement is also obtained with the ideal linear survival curve with a potential survival time of 113 days (curve 3).

### Reference Range for Red Cell Survival Times with Correction for $^{51}$Cr Elution Using Dornhorst Method

The mean red cell survival time corrected for elution for reference subjects was 113.0 ± 32.5 days with a 95% confidence range for normal subjects of 47–179 days for the Dornhorst method and 109.3 ± 25.2 days with a confidence range of 58–161 days for the weighted-mean method. The estimated red cell survival times for the weighted-mean method and the Dornhorst function were not different.

### Correction for Elution Using the Dornhorst Rate Constant Method

Red cell survival time, with correction for elution applied by using the Dornhorst rate constant method, was 235 ± 221 days. This method of calculating red cell survival time corrected for elution resulted in unreliable

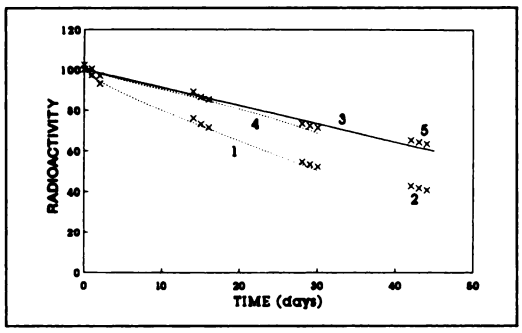


**FIGURE 3.** Good agreement was obtained between the ICSH published survival curve not corrected for $^{51}$Cr elution (Curve 1: . . .) and the mean uncorrected survival curve measured in this study (Curve 2: x). The ideal survival curve (Curve 3: _) given by: 100(1-t/113), the ICSH survival curve (Curve 4: . . .) and the mean survival curve in this study (Curve 5: x), corrected for elution, were in agreement during the initial 30 days.

results due to the large variation in the value of the removal rate of radioactivity $k_T$ (0.0157 ± 0.0062 $day^{-1}$). The estimate of the potential survival time T (3,894 ± 146,178 days) was clearly also not within a physiologic range. Additional data points up to the survival time of the red cells are required to calculate these parameters accurately. This would require blood sampling for a period of up to 133 days. This was not possible using volunteers.

## CONCLUSION

This study shows that red cell survival time can be calculated using the weighted-mean or Dornhorst functions with personal computers. The correction factors based on the Dornhorst function gave acceptable results for calculating red cell survival time corrected for elution. Reference red cell survival times were presented for the weighted-mean and Dornhorst methods with and without correction for the elution of $^{51}Cr$.

Moreover, this study shows that the influence of chromium elution is often underestimated as result of substantial variations in elution rate from subject to subject. Cline and Berlin (*9*), Garby and Mollison (*10*), as well as Bentley (*8*) found a coefficient of variation of 37%, 36% and 25%, respectively, for chromium elution in patients and normal subjects (Table 1). We did not experimentally measure elution rate by comparing chromium survival curves with survival curves obtained with labels that do not elute from red cells. However, the rate constant $k_T$ obtained in this study in normal subjects also reflects the variation in elution rate. We found a coefficient of variation of 39%. If the red cell life span is near normal, correction for elution becomes important. Variations in elution rates will then significantly influence the accuracy of estimated red cell survival times.

We could not recommend the calculation of red cell survival time using the Dornhorst rate constant correction method to correct for elution. Nonphysiologic results were obtained with this method as result of the substantial variation in the elution rate of chromium from the red cells. Also, as a result of rapid elution, data points over a longer time span are required that are not practical in reference subjects or patients with near normal survival times.

Improved methods for analysis of red cell survival curves, based on the Dornhorst function, were evaluated in this investigation. The accuracy of the measurement of red cell survival was not improved due to the variable elution rate of chromium from red cells. Maximal benefit of the proposed methods for data analysis will be derived if a label can be developed with a smaller and constant elution rate. Also, these methods will be of value in dual-label studies for the compatibility of transfused blood, where an estimate of chromium elution is measured in each subject.

We recommend the use of the Dornhorst function and weighted-mean method to calculate red cell survival time. Moreover, the weighted-mean method is recommended if computing facilities are not available.

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