# Radio-isotope Red Cell Survival Studies Recommended by ICSH*

## 放射性同位元素를 利用한 赤血球壽命測定法(ICSH 推薦)

血液疾患, 특히 溶血性貧血을 隨伴한 境遇에 赤血球의 生成 및 破壞過程을 正確히 把握하는 것은 重要하며 특히 赤血球壽命測定은 貧血의 本態 및 發生機轉을 理解하는데는 물론 病因的 治療 및 豫後를 決定하는데 大端히 有用하다. 赤血球壽命測定에는 1919年 Ashby가 開發한 differential agglutination法이 利用되어 왔으나 輸血에 따른 危險이 있고 方法이 複雜하다는 短點을 가져 새로운 赤血球壽命測定法이 硏究되어 왔다. 最近에 $^{51}Cr$이나, $^{32}DFP$ 같은 放射性同位元素를 利用한 方法이 導入된 以來, 臨床的으로 赤血球壽命測定이 많이 施行되고 있지만 아직까지도 그 方法이나 結果의 解釋에 標準化가 안 되어 있다. 現在 臨床領域에서 가장 널리 利用되고 있는 赤血球壽命測定法은 放射性 chromium($^{51}Cr$)法으로 1950年 Gray와 Sterling에 依해 創案된 以來 많은 學者들에 依해 여러가지 變法이 考按되어 왔는데, 이의 가장 큰 理由는 $^{51}Cr$이 赤血球標識에 가장 理想的인 것만은 아니고 그 結果에 많은 要因들이 影響을 미치기 때문이다. 또 이런 變法의 使用은 各 檢査에서 計算된 測定値에 差異가 있어 그 結果의 解釋 및 比較 檢討에 적지않은 難點이 생겨 標準化된 共通的인 方法의 使用이 重要하다는 事實이 認識되게 되었다. 1966年 濠洲의 Sydney에서 開催되었든 第11次 國際血液學會때 열린 第4次 International Committee for Standardization in Haematology(ICSH)에서 Diagnostic Applications of Radioisotopes in Haematology에 關한 expert panel을 갖을것을 議決하여 다음과 같은 12名의 委員이 決定되었으며 委員會의 議長에 Dr. Szur, 總務에 Dr. Glass가 各各 選任되었다. 그間 1967年 英京 London에서 첫 會合이 있은後 New York, Vienna(IAEA 後援) Bethesda(NIH 後援)에서 專門委員會를 갖고 赤血球壽命測定法에 關한 意見의 一致를 보았다.

ICSH와 國際血液學會에서는 이번에 決定된 赤血球壽命測定法을 널리 紹介하며, 測定法과 얻어진 結果의 解釋에 標準化를 期할 目的으로 이에 聯關性있는 專門誌에 揭載할 것을 要請 받었기에 이에 全文을 紹介하는 바이다.

이들은 放射性·chromium法의 모든 細部的인 面을 標準化하고 있으며 그間 가장 論難의 對象이 되었던, $^{51}Cr$-標識方法에 있어서의 세가지 變法, 즉 ACD法, Citrate-wash法, ACD/ascorbic acid法을 모두 認定하고 있다. 또한, DFP($DF^{32}P$ 또는 $^{3}H$-DFP) 標識法의 標準方法도 記述하고 있으며, 더욱 重要한 것은 赤血球平均壽命算出法으로서 現在까지 大部分의 學者가 使用하여 왔던 指標인 $T_{50}Cr$ 代身에 mean red cell life span을 使用할 것을 勸하고 있다. (編輯者註)


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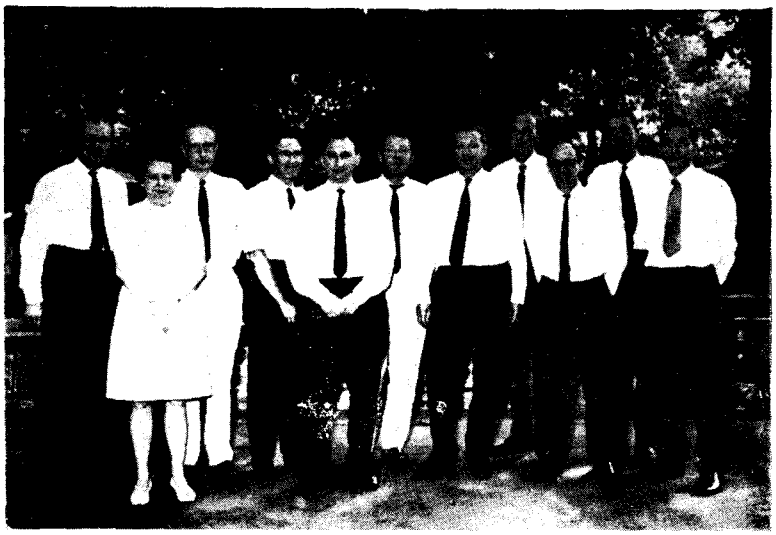

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The Fourth General Assembly of the International Committee for Standardization in Haematology, which met during the 11th International Congress of Haematology, held in Sydney, Australia, 1966, established an expert panel on the Diagnostic Applications of Radioisotopes in Haematology. The panel comprised E.H. Belcher(I.A.E.A.), N.I. Berlin,(U.S.A.), J.G. Eernisse, (Netherlands), L. Garby, (Sweden), H.I. Glass, (U.K.), H. Heimpel, (Federal Republic of

Germany), M. Lee,(Republic of Korea), S.M. Lewis, (U.K.), P. McIntyre, (U.S.A.), P.L. Mollison, (U. K.), Y. Najean, (France), and L. Szur, (U.K.). The chairman of the panel was Dr. Szur and the secretary Dr. Glass. The panel held its inaugural meeting in London, 1967, and decided to deal first with estimation of red cell survival and associated investigations. Subsequently, the Committee met on three occasions, in New York, in Vienna (under the auspices of the International Atomic Energy Agency) and in Bethesda (under the auspices of the National Institutes of Health). The following document deals with red cell survival studies. It is hoped that in the near future, similar documents will be issued dealing with other applications of radioisotopes in haematology.

Red cell survival studies are performed in large numbers in hospitals throughout the world but so far there has been no standardization, either of methods or of interpretation of results. The present document deals with only some of the methods available.

The methods of red cell labelling which are used fairly widely in clinical practice are those of 'random labelling' in which either the whole population of red cells, or a sample of the whole population, is labelled. Techniques in which only red cells produced over a very limited period of time are labelled ('cohort labelling') have so far been used only for research purposes.

For random labelling, radioactive sodium chromate has been more widely used than any other label. In describing results observed with radiochromium, most workers have until now used a single index: the time taken for half the label to leave the circulation, commonly known as $T_{\frac{1}{2}}$ Cr or, better, the $T_{50}$Cr. Because chromium elutes from red cells and because the curves of disappearance of red cells from the circulation may have more than one component, the $T_{50}$Cr has no simple relationship to the mean red cell life span, which is the parameter required in clinical practice.

The situation is further complicated by the fact that values for the $T_{50}$Cr in normal subjects vary considerably. This variability is due partly to the use of different techniques of labelling, which have been shown to affect the rate of elution of chromium from the red cells, and partly to differences in calculating the results.

The present document sets out to standardize all details of the radiochromium method, although three minor variations in the method of Cr-labelling are allowed.(Table Ⅲ) The more important methods of deducing mean cell life from radioactive chromium measurements are described. It is hoped that the use of the index "$T_{50}$Cr" will before long be abandoned.

Standard methods for labelling red cells with DFP (both $DF^{32}P$ and $^{3}H$-DFP) are also described. DFP has the great advantage of not eluting from red cells (except during the first 24 hours, when most observers find some loss).

Throughout the document, unless otherwise specified, it is assumed that the patient's own red cells are being labelled. Donor red cells may be needed to study compatibility (see section 4) and are also needed very occasionally to discover whether a haemolytic state is due to an intrinsic defect of the patient's red cells or to some pathological process resulting in the accelerated destruction of intrinsically normal red cells. Except in these circumstances survival studies should be conducted using the patient's own red cells, to avoid the risk of iso-immunization and of transfering infectious disease.

## STANDARD TECHNIQUES FOR RED-CELL SURVIVAL STUDIES

### 1. Techniques using $^{51}$Cr

#### 1.1 Labelling

During labelling, all operations should be carried out by sterile techniques and all solutions used must be sterile and pyrogen free. The following description allows one of three methods to be used. In the most commonly used of these, method A (ACD method), blood is mixed with acid-citrate-dextrose (ACD) and some of the supernatant citrate plasma is removed before incubation with $^{51}$Cr. The red cells are washed before being injected. In method B (citrate-wash method) the red cells are washed in citrate-phosphate-dextrose solution before being incubated with $^{51}$Cr; in other respects this method is exactly the same as

method A. In method C (ACD/ascorbic acid method) the red cells are not washed following incubation with $^{51}Cr$ but instead ascorbic acid is added and the suspension injected directly.

1.1.1 Methods A and C: Obtain blood in a plastic or glass syringe by venepuncture. Add 10 volumes of blood to 1.5 volumes of "N.I.H.A" ACD solution.* (Other formulae do not appear to have obvious physiological advantages. The commercial availability of pre-packed sterile kits for $^{51}Cr$ labelling of red cells does have practical convenience; however, their use may require different factors for elution correction (See 3.3).

Method B: Obtain blood by venepuncture and add one volume of blood to not less than two volumes of citrate-phosphate-dextrose sotution.**

1.1.2 Methods A and B: Centrifuge the suspension at 1000~1500g for 5~10 minutes. Remove and discard supernatant plasma taking care not to remove any red cells. If leukocyte count is greater than 25,000/$\mu$l also remove and discard buffy coat.

Method C: Centrifuge suspension only if leukocyte count exceeds 25,000/$\mu$l. In this event, following centrifugation, remove and set aside plasma. Remove and discard most of buffy coat, taking care to remove as few red cells as possible. Replace plasma and mix well.

1.1.3 Add $^{51}Cr$ sodium chromate solution slowly and with continuous gentle mixing to the packed red cells or blood. The amount of $^{51}Cr$ added should be as low as possible consistent with the equipment used to measure the radioactivity of the samples. In no case should the activity of the injected $^{51}Cr$ be greater than 1.5 $\mu$Ci/kg body weight. The specific activity of the $^{51}Cr$ should be such that less than 2 $\mu$g of chromium is added per ml of packed red cells. The added $^{51}Cr$ sodium chromate should be in a volume of at least 0.2 ml, being diluted in 9 g/l sodium chloride solution (isotonic saline). Allow mixture to stand at room temperature for 15 minutes or incubate in a water bath at 37°C for this period.

1.1.4 Methods A and B: Wash labelled cells twice in 4~5 volumes of isotonic saline. After the second wash there is generally less than 1% of the remaining radioactivity in the supernatant liquid. Resuspend red cells in a sufficient volume of isotonic saline to allow intravenous injection of 20 ml, or a lesser amount if appropriate to a particular circumstance (e.g. children). When red cell osmotic fragility is greatly increased e.g. in some cases of hereditary spherocytosis, wash the red cells in 12 g/l sodium chloride solution.

Method C: Add 50 mg of ascorbic acid and mix well.

1.1.5 If the total red cell volume or external blood loss is to be determined, set aside an aliquot of the labelled red cell suspension for preparation of appropriate standards. When method C is used it is necessary to determine the packed cell volume(PCV) of this aliquot and centrifuge part of it so that the amount of radioactivity in the plasma can be determined. Inject a known amount of the labelled red cell suspension. Methods to determine this amount precisely will be discussed in the document on standard techniques for estimation of blood volume.

**1.2 Sampling**

At 10 minutes take a 5~10 ml blood specimen from a vein other than that used for injection. When it is suspected that mixing will not be complete in 10 minute (e.g. in patients with gross splenomegaly) a specimen should be taken at 60 minutes instead. Solid heparin (0.1 mg/ml of blood) or ethylene-diaminetetraacetate (EDTA)(1.5±0.25mg/ml of blood) should be used as anticoagulant. When method C is used, it is necessary to centrifuge part of the 10 or 60 minute specimen so that the amount of radioactivity in the plasma can be determined and an appropriate correction applied. Take a further blood specimen at 24 hours, 3 further specimens between day 2 and day 7

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* "N.I.H.A" ACD solution:

| | |
|---|---|
| Trisodium citrate dihydrate | 2.0 g |
| Citric Acid | 0.8 g |
| Dextrose | 2.5 g |
| Water to | 100 ml |

** Citrate-phosphate-dextrose solution:

| | |
|---|---|
| Trisodium citrate dihydrate | 3.0 g |
| Sodium dihydrogen phosphate | 0.015 g |
| Dextrose | 0.2 g |
| Water to | 100 ml |

The solution has a pH of 6.9 after autoclaving at room temperature and has a brown tinge.

as a check on the stability of the measuring equipment. A sufficient amount of this standard solution should be prepared to allow aliquots to be discarded after measurement. Its activity should be comparable with that of the 60-minute blood specimen.

2.3.2 Dry-sample counting:Disposable tray method ($DF^{32}P$)

Centrifuge approximately 5 ml of each blood specimen. Remove and discard supernatant plasma. Wash cells three times with saline as described above. Make up red cell suspension approximately to the original volume and measure the PCV. Mix suspension well and pipette 1 ml samples into disposable trays of aluminium foil. Dry samples at approximately 70°C. Seal each tray in cellophane. For radioactivity measurement, wrap the tray around a cyclindrical thin-wall Geiger-Müller counter tube.

2.3.3. Dry-sample counting: Planchet method ($DF^{32}P$)

Centrifuge approximately 10ml of each blood specimen. Wash cells three times with saline as described above, resuspend cells and measure the PCV of the suspension and then lyse cells with saponin. As the sample is to be dried later, the saponin may be added in an equal quantity of water. Alternatively, lysis can be achieved by adding an equal quantity of 10 g/l ammonium hydroxide solution. Pipette known volumes of lysate on to metal planchets to the floors of which discs of absorbent material have been fixed. Dry samples at a temperature not exceeding 30°C. Measure radioactivity of dried samples by means of gas-flow or thin-window Geiger-Müller counter.

2.3.4. Combustion/liquid scintillation counting method ($^3$H-DFP)

Centrifuge approximately 2 ml of each blood specimen. Wash cells 3 times with saline as described above. Lyse cells by the addition of an equal volume of distilled water. Dry lysate to constant weight at 70°C and grind residue to a powder. Combust a known weight of this powder in a Schöniger apparatus or other combustion apparatus that yields water quantitatively. Transfer resulting $^3$H-water to a counting vial containing liquid scintillator. Measure radioactivity of samples in a liquid scintillation counter.

## 3. Presentation and Analysis of Red Cell Survival Data

3.1. The object of red cell survival studies is to obtain estimates of the rates of red cell production and destruction. If the patient is in a steady state (see 3.2) the rate of destruction (and, by definition, the rate of production) may be obtained as the product of the volume (or number) of circulating red cells and the fractional rate of disappearance of labelled cells at zero time. The reciprocal of this latter value is, by definition, equal to the mean red cell life span. Mean red cell life span is here defined as the mean survival time of all circulating red cells irrespective of whether they are destroyed by random destruction or senescence mechanisms. Derivation of red cell production and destruction is simple only when the patient is in a steady state and when the red cell label is not eluted from the circulating red cells.

It is common to use $^{51}$Cr survival data to determine the $T_{50}Cr$, that is, the time taken for the concentration of $^{51}$Cr in the circulating blood to fall to 50% of its initial value after correction of the data for physical decay. The chief objection to the use of this index is that without additional information, mean red cell life span cannot be calculated from it. Since it is preferable to derive estimates of mean red cell life span from the data, methods of doing this will be described, and methods of estimating "$T_{50}Cr$" will not be discussed.

3.2. A patient is in a steady state when the rates of production and destruction of red cells have been constant for a time that is not less than the life span of the longest living cells in the particular patient. "Destruction" in this context includes the loss of red cells from the circulation by haemorrhage, both internal and external. In practice, a patient can be considered to be in a steady state if during the previous two months and throughout the period of the study, the reticulocyte count and the haemoglobin concentration (or PCV) do not change significantly and the patient is not being transfused. Information on the haematological indices during the period pre-

and thereafter at least 2 further specimens per week for the duration of the study. Measure the haemoglobin (g/100 ml) by the hemiglobincyanide (cyanmethaemoglobin) method or the PCV on a part of the specimen.

**1.3 Sample preparation and radioactivity measurement**

For the preparation of samples for the measurement of radioactivity add to each specimen a small amount of saponin powder and mix well, preferably on a mechanical rotary mixer for 5 minutes. Deliver 1~3 ml of the lysed blood into a counting tube. In cases of polycythaemia and in case where the blood is unusually viscous the whole blood should be well mixed and delivered in the the counting tube before being lysed with saponin. Agitate gently, taking care not to allow the sample to touch the cap of the counting tube. The volume delivered must be known precisely and if necessary made up to the same volume in each consecutive sample. To achieve precision and to minimize errors in pipetting, selected pipettes of similar type should be used for a given series of samples. Alternatively, pre-calibrated all-glass 1 ml tuberculin syringes may be used. When practical it is preferable to prepare replicate samples of each specimen.

1.3.1 Measure the radioactivity of each sample in a well-type scintillation counter or other suitable gamma ray measurement system to a statistical accuracy of ±2% (1 standard deviation). When method C is used the 10 minute (or 60 minute) plasma sample must also be measured, but a lower statistical precision is acceptable for this sample.

## 2. Techniques using radioactive diisopropyl phosphorofluoridate (DFP)

**2.1 Labelling**

Labelling may be carried out *in vivo* or *in vitro* using either $^{32}$P-DFP (DF$^{32}$P) or $^{3}$H-DFP. Labelling *in vivo* is carried out by intravenous injection of DFP in propylene glycol. Intravenous injection is preferable to intramuscular injection because of a lesser degree of early elution. *In vitro* labelling has the disadvantage that the low uptake of DFP necessitates the injection of a large volume (100~200 ml) of labelled cells.

2.1.1 Methods

Inject the radioactive DFP solution intravenously over a period of 10~15 minutes. The amount of DFP injected should not be greater than 0.02 mg/kg body weight. The activity of the radioactive DFP injected should not exceed 0.7 μCi/kg body weight with DF$^{32}$P, or 7 μCi/kg body weight with $^{3}$H-DFP. If the radioactive DFP solution has to be diluted, this should be done immediately before injection using not more than 10 ml of isotonic saline.

**2.2 Sampling**

At 60 minutes take a blood specimen from a vein other than that used for injection. Solid heparin (0.1 mg/ml blood) or EDTA (1.5±0.25mg/ml blood) should be used as anticoagulant. Take a further blood specimen at 24 hours, 3 further specimens between day 2 and day 7 and thereafter at least two further specimens per week for the duration of the study. Measure the haemoglobin or PCV on each specimen. The volume of blood removed must be appropriate to the method to be used for radioactivity measurements.

**2.3 Sample preparation and redioactivity measurements**

Three methods of sample preparation for DF $^{32}$P and one for $^{3}$H-DFP are described. The choice of method for DF$^{32}$P depends on the laboratory facilities available.

2.3.1 Liquid-sample counting method (DF$^{32}$P)

Mix each blood specimen well and pipette a known amount (usually 10 ml) into a tube with a "10 ml" mark. Centrifuge blood. Remove and discard supernatant plasma. Wash red cells 3 times with isotonic saline (but see 1.1.4). Care must be taken not to remove any red cells during these procedures. After the final wash lyse cells by adding a small amount of saponin powder and add saline to the 10 ml mark.

Measure the radioactivity of the lysed samples in a liquid sample Geiger-Müller counter tube designed to accept approximately 10 ml of liquid. It is desirable to measure an aliquot of a standard solution containing $^{32}$P ($^{32}$P-phosphate or hydrolysed D$^{32}$P in 10 g/l sodium phosphate solution) after every few samples

ceding the study may however be incomplete in which case a steady state may have to be assumed. Measurements of radioactivity in blood samples derived from patients in a steady state should be expressed as counting rate/g Hb, or counting rate/ml red cells or counting rate/ml of whole blood. In a non-steady state it is not possible to measure mean red cell life span with accuracy partly because the age distribution of red cells in a population may be distorted and partly because the red cell volume is changing. Nevertheless on the assumption that the total blood volume remains constant an approximate estimate can be obtained if the measurements are expressed as counting rate per ml of whole blood.

3.3. $DF^{32}P$ is an especially satisfactory label because, at least after the first 24 hours, it is not eluted from the red cells. On the other hand $^{51}Cr$ is eluted at a rate which significantly affects estimates of mean red cell life span. The average rate of chromium elution is of the order of 1% per day, which is of the same magnitude as the normal rate of red cell destruction. Accordingly, variations in the rate of elution in different individuals may seriously affect the accuracy of estimates of mean red cell life span when survival is normal or only slightly reduced. On the other hand, when mean red cell life span is considerably reduced, chromium elution and variations in the rate of chromium elution become relatively unimportant.

3.4. The following sections summarize the way in which mean red cell life span should be estimated from $DF^{32}P$ or $^{51}Cr$ measurements in a steady state. Correction for physical decay is assumed.

3.4.1. In studies with $^{51}Cr$ in which method C is used, correct the measurements on the 10 or 60 minute sample for plasma radioactivity. Correct all $^{51}Cr$ measurements for elution (see Table 1).

3.4.2 Plot the data, whether derived from $^{51}Cr$ (corrected for elution) or from DFP, as counting rate/g Hb, counting rate/ml red cells or counting rate/ml whole blood on semi-logarithmic and linear graph paper.

3.4.3 Examine the plot on linear paper to see if a straight line can be fitted to the data points. If so, use a least squares fitting procedure to obtain the line, and determine the goodness of fit. If a straight line does not fit the data well, examine the plot on semi-logarithmic paper to see if a straight line can be fitted to the data thus plotted. If so, again use a least squares fitting precedure to obtain the line and determine the goodness of fit. If it is not obvious by simple inspection which plot gives the best fit apply a statistical fitting criterion. If neither of these procedures results in all the data being satisfactorily fitted by a

**Table I Mean Cr survival in normal subjects and correction factors which convert the Cr survival into 'true' red cell survival(mean red cell life-span 115 days), when the citrate-wash method (Method B) is used.**

| Day | Cr. Survival | Correction Factor |
|---|---|---|
| 0 | 100.0 | |
| 1 | 96.2 | 1.03 |
| 2 | 94.0 | 1.05 |
| 3 | 92.0 | 1.06 |
| 4 | 90.1 | 1.07 |
| 5 | 88.2 | 1.08 |
| 6 | 86.5 | 1.10 |
| 7 | 84.7 | 1.11 |
| 8 | 83.1 | 1.12 |
| 9 | 81.4 | 1.13 |
| 10 | 79.9 | 1.14 |
| 11 | 78.3 | 1.16 |
| 12 | 76.7 | 1.17 |
| 13 | 75.2 | 1.18 |
| 14 | 73.7 | 1.19 |
| 15 | 72.2 | 1.20 |
| 16 | 70.7 | 1.22 |
| 17 | 69.3 | 1.23 |
| 18 | 67.8 | 1.25 |
| 19 | 66.3 | 1.26 |
| 20 | 64.9 | 1.27 |
| 21 | 63.4 | 1.29 |
| 22 | 62.0 | 1.31 |
| 23 | 60.5 | 1.32 |
| 24 | 59.1 | 1.34 |
| 25 | 57.6 | 1.36 |
| 26 | 56.2 | 1.38 |
| 27 | 54.7 | 1.40 |
| 28 | 53.3 | 1.42 |
| 29 | 51.9 | 1.45 |
| 30 | 50.4 | 1.47 |

**Table Ⅱ Radiation dose to the patient in red cell survival studies**

| Label | Critical organ | Fraction of administered radioactivity reaching critical organ | Mean radiation dose to patient per μCi administered (mrad) |
|---|---|---|---|
| $^{51}$Cr (a) | Spleen | 0.2 | 3.87 |
| $^{51}$Cr (b) | Spleen | 1.0 | 89.3 |
| DF$^{32}$P (c) | Blood | 0.25 | 27.7 |
| $^{3}$H-DFP(c) | Blood | 0.25 | 3.52 |

Notes:
(a) Intravenous injection of labelled normal compatible cells into normal subjects
(b) Intravenous injection of labelled damaged or non-compatible cells
(c) *In vivo* labelling of cells by intravenous injection of label

straight line, obtain the initial slope of the curve on the linear plot by drawing a straight line through the first few points only. In studies with DFP, the 60 minute data point should not be used in the fitting procedures, since some elution of this label may occur over the first 24 hours.

3.4.4 Derive the mean red cell life span as the reciprocal of the slope of the straight line obtained by one of the three procedures outlined in 3.4.3. In the case of a semi-logarithmic plot, the reciprocal of the slope is obtained by multiplying the half time of the fitted line by 1.4.4

3.5 If computing facilities are available the following method is used instead of that described in 3.4.3 and 3.4.4:

3.5.1 Fit functions (1) and (2) below to the data:

$$N_t/N_o=(e^{-kt}-e^{-kT})/1-e^{-kT} \quad (1)$$

$$N_t/N_o=\alpha e^{-\beta t}+(1-\alpha)e^{-\gamma t} \quad (2)$$

Where $N_t$=radioactivity at time "t" corrected if necessary for elution and expressed in any of the ways described in 3.1.1

$N_o$=radioactivity at time "0" corrected if necessary for elution and similarly expressed.

Equation (1) is based on a model for red cell survival and assumes that all the circulating red cells have the same potential life span (T) and are subject to random destruction at a constant rate with a coefficient (k). Estimation of the mean cell life span is however independent of whether the model is correct or not.

Equation (2) is one of several empirical formulae which can be used simply for fitting a mathematical function to the data. The constants $\alpha,\beta,\gamma$ are purely empirical and have no physiological significance.

3.5.2 If function (1) fits the data better, use the initial slope of the fitted function to obtain an estimate of the mean red cell life span. In this case the

**Table Ⅲ**

| Procedure | Method A | Method B | Method C |
|---|---|---|---|
| 1. Anticoagulant | A.C.D. | C.P.D. | A.C.D. |
| 2. Sample used for labelling | Red-cell suspension (after centrifugation) | Red-cell suspension (after centrifugation) | Whole blood (Remove buffy coat only if wbc<25000/μl) |
| 3. Isotope addition | Identical for each Method | | |
| 4. Removal of unbound chromium | Washing in saline | Washing in saline | Addition of ascorbic acid |
| 5. Standard (for blood volume detection) | Aliquot of washed cell suspension | Aliquot of washed cell suspension | Aliquot of blood corrected for plasma |
| 6. Sampling | Identical for each Method | | |
| 7. Sample preparations 10'and 60' samples | Lysed whole blood | Lysed whole blood | Lysed whole blood: corrected for plasma radioactivity |
| 8. Subsequent samples | Identical for each Method | | |

computer derived parameters T and k also yield estimates of the potential life span and coefficient of random destruction.

3.5.3 If function (2) fits the data better, use the initial slope of the fitted function to obtain estimate of the mean red cell life span only.

3.6 In cases where there is an external blood loss and where this blood loss has been constant for a long time, the true mean red cell life span (T) is given by the equation below, where $T_a$, the apparent mean red cell life span is calculated in accordance with 3.4.2—3.5.3

$$\bar{T}=T_a \cdot \frac{V}{(V-T_aL)}$$

where $\bar{T}$=true mean red cell life span (days)
$T_a$=apparent mean red cell life span (days)
V=total red cell volume (ml)
L=average rate of loss of red cells (ml/day)

## 4. Use of isotope labelled red cells as a test of compatibility

**4.1 The main indication for the test are as follows:**

(a) When serological tests suggest that all donors are incompatible. (b) when 'cold' antibodies are present, active *in vitro* at 30°C or higher and a non-reacting donor cannot be found, (c) when the recipient has had an unexplained hemolytic transfusion reaction and requires a further transfusion.

**4.2 Conduct of test**: Take a blood sample from a potential donor, label approximately 0.5 ml of red cells with 20 $\mu$Ci $^{51}$Cr and make up a suspension of the washed cells to about 13 ml. Inject 10 ml and prepare a standard from the remainder. Note the time of injection of the cells precisely by starting a stop-watch at the mid-point of injection. Take blood samples at 3′, 10′ and 60′ from a vein other than that used for the injection. At 10′ and 60′ take sufficient blood to provide plasma as well as whole blood for radioactivity measurement.

Preferably, estimate the recipient's red cell volume approximately either by injecting a sample of the recipient's own red cells labelled with a small amount of $^{51}$Cr (10 $\mu$ Ci) before injecting the donor red cells (in this case labelled with 50 $\mu$Ci) or by injecting the recipient's own red cells labelled with $^{32}$P. Alternatively, deduce the recipient's red cell volume from the patients weight, height and PVC.

**4.3 Interpretation**: When compatible red cells have been injected, the counting rate of the 60 minute sample is, on the average, about 99% of that of the 3 minute sample. In the individual case, due to errors of measurement, values between 94% and 104% may be accepted as normal.

In cases of urgency or when there is great difficulty in finding completely compatible red cells, donor red cells may be transfused with minimal hazard when, following a test with 0.5ml of the donor's red cells, the amount of radioactivity in the plasma, both at 10 and 60 minutes, does not correspond to more than 5% of the radioactivity injected and when red cell survival at 60 minutes is not less than 70%. (N.B. if survival is at least 70% the deduction is that the concentration of the offending antibody is very low so that the destruction of a large volume of incompatible red cells will be either negligible or will take place only slowly).

## 5. Radiation Dose to the Patient in Red Cell Survival Studies

5.1 Table Ⅱ gives the data relating to the radiation doses to a 70 kg patient in the procedures described above. It should be emphasized that the calculations on which these data are based involve many assumptions. Firstly, the blood circulation cannot readily be represented in terms of a physical model for purposes of dose calculation. Secondly, detailed quantitative data concerning the distribution and fate of the different radioisotopic labels in the body are lacking. Thirdly, even if such data were available for a given procedure in normal subjects, the corresponding data in pathological conditions could be very different. The data do not therefore give more than an approximate indication of the radiation dose to the patient in the various procedures.

5.2 Table Ⅱ shows the dose to the critical organ or tissue—the organ or tissue of the body most likely to suffer radiation damage as a consequence of the procedure in question—per $\mu$Ci of administered radioactivity.

5.2.1 When $^{51}Cr$-labelled red cells are injected, $^{51}Cr$ is slowly eluted from the cells in the circulation. The labelled cells eventually undergo destruction in the reticulo-endothelial tissues, mainly in the spleen, from which the deposited radioactivity is again slowly eluted. In these circumstances, the critical organ may be taken as the spleen. Two extreme situations are considered. The first of these corresponds to a normal red cell survival study; the labelled cells are assumed to be removed from the circulation at the end of their normal life span, 20% being trapped in the spleen. The second corresponds to a study with damaged or non-compatible cells; the labelled cells are assumed to be rapidly removed from the circulation, 100% being trapped in the spleen. The dose to this organ is then, of course, considerably higher.

5.2.2 When cells labelled with radioactive DFP are injected, elution of the label in the circulation is insignificant after the first 24 hours. The labelled cells again eventually undergo destruction in the reticulo-endothelial tissues, notably in the spleen, but in this case the deposited radioactivity is quite rapidly lost from the tissues. In these circumstances, the critical organ may be taken as the circulating blood itself. Only the situation corresponding to a normal red cell survival study is considered here, since this case will result in the highest radiation dose to the circulating blood. With $DF^{32}P$ the radiation dose is considered to be delivered to the whole blood, with $^{3}H$-DFP to the red cells only.