SHORT-TERM ASSESSMENT OF RED BLOOD CELL SURVIVAL USING ^{51}Cr

BY

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(RECEIVED FOR PUBLICATION APRIL 19, 1960)

^{51}Cr techniques for the study of red blood cell survival over long periods in the steady state are now well established (Ebaugh, Emerson, and Ross, 1953; Mollison and Veall, 1955; Hughes Jones and Mollison, 1956). This paper presents the results of an investigation of the validity of a ^{51}Cr study not exceeding 10 days which is well suited to a combination with a ^{59}Fe technique for the rapid simultaneous assessment of red cell production and destruction where haematological conditions may show fairly rapid changes.

Material and Methods

Ten normal subjects and 12 patients with normal ^{51}Cr red cell survival were investigated. Twenty millilitres of the subject's blood, withdrawn into heparin, was centrifuged at 1,800 r.p.m. for 15 minutes. The plasma was removed and 100 μc . ^{51}Cr as $\text{Na}_2^{51}\text{CrO}_4$ added to the packed cells. After standing at room temperature for 30 minutes the cells were washed once, suspended in isotonic saline, and then reinjected into the subject. A blood sample was taken 15 minutes after reinjection for determination of blood volume, and afterwards about 12.5 ml. 0.1% sodium citrate in isotonic saline containing 10 μc . of $^{59}\text{FeCl}_3$ was given intravenously to allow the subsequent determination of plasma ^{59}Fe clearance and turnover, the utilization of ^{59}Fe for red blood cell production, and the ^{59}Fe surface counting pattern (Wetherley-Mein, Hutt, Langmead, and Hill, 1956). ^{51}Cr activity was determined in blood samples taken in a standard sequence (disposition A, Table I) during 10 days following reinjection of the tagged cells.

The component ^{51}Cr and ^{59}Fe activities in blood samples containing both isotopes were determined in a well-type scintillation counter, the scaler being equipped with a variable voltage discriminator, thus allowing the inclusion or exclusion of ^{51}Cr activity in the total counting rate.

Determination of Rate of ^{51}Cr Loss.—Starting 48 hours after the injection of ^{51}Cr cells the values representing ^{51}Cr activity over seven to 10 days are plotted logarithmically against time on a linear scale and the best-fitting straight line through the points is drawn. From the slope of this line the rate ^{51}Cr loss (percentage loss per day) may be calculated. It

is convenient to calibrate a protractor in terms of daily percentage ^{51}Cr loss against a standard time scale.

Results

From these pooled residual error variances, we estimated the overall error of a single careful determination of relative ^{51}Cr activity to correspond to a coefficient of variation of $\pm 2.85\%$; of this we could identify a quantity estimated as $\pm 1.91\%$ as referable to the actual counting, which we termed "scaler error." The residual difference ($\pm 2.15\%$) thus represents all other handling errors (Ingram, Langmead, and Jones, to be published).

We examined two series of results in the normal subjects and patients with no evidence of shortened red cell survival and found no evidence of any real differences in the ^{51}Cr activity of serial blood samples other than those assignable to the steady removal of the isotope from the blood. This investigation also confirmed the empirical justification for the expression of normal ^{51}Cr loss in exponential terms over short periods of time (see Discussion). In 10 normal subjects we obtained a mean rate of loss of ^{51}Cr from the circulation of 2.99% per day, with the real variation between subjects giving a coefficient of variation of $\pm 30\%$. Our mean rate agrees well with the figure of about 2.75% per day derived from the data for days 2 to 10 recorded by Mollison and Veall (1955) in 11 normal subjects. We also found that observed short-term fluctuations in the background activity, i.e., within a

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day, did not detectably contribute to our experimental errors.

From these analyses we were able to compute the critical values for the least rate of ^{51}Cr loss which could confidently be regarded as abnormal when derived from various dispositions of blood samples in a 10-day period. These values are given in the right-hand column of Table I and

TABLE I
DISPOSITIONS OF SAMPLES

	Disposition of Blood Samples									Limiting Value for ^{51}Cr Slopes (% Loss of Activity per Day)		
	Days after Injection of ^{51}Cr (Day 0)											
	2	3	4	5	6	7	8	9	10			
(A)	x	x	x				x			x	x	5.1
(B)	x	x	x				x	x	x			5.4
(C)	x	x	x			x	x	x				5.6
(D)	x	x	x		x	x	x					5.7

x indicates that a blood sample should be taken.
x x indicates that two separate samples should be taken, one immediately after the other, from different veins.

show the least slope which for each disposition of samples defines the lower limit of abnormal ^{51}Cr loss with the same confidence as 5.1% per day in our standard 10-day sequence (disposition A). On this basis, a similar grouping of six samples over six days (disposition C) must yield a rate of ^{51}Cr loss greater than 5.6% per day to be regarded as abnormal.

As with the measurement of any constant rate of change, ^{51}Cr loss is progressively more accurately determined as the time over which it is observed is lengthened. Within a given period the accuracy is also affected by the disposition in time of the readings obtained since its precision is governed by the moments of these readings about the midpoint. Thus, for a given number of blood samples over 10 days, accuracy is improved by bunching the samples at each end of this period.

The gain in accuracy obtained by bunching the samples at the ends of the study is seen by comparing dispositions B and D.

The left-hand column in Table I gives three dispositions of samples found useful in clinical situations where blood samples cannot be taken in the standard 10-day sequence (A).

Discussion

In normal subjects correction of the ^{51}Cr survival curve for a uniform rate of chromium elution from red cells fails to give the linear slope that is taken to represent true normal cell survival (Hughes Jones and Mollison, 1956). This causes

difficulty in interpreting survival curves that do not differ greatly from normal. One might use the early part of the curve to obtain an estimate of mean cell life (Dornhorst, 1951; Hughes Jones and Mollison, 1956). However, since the "early loss" of ^{51}Cr makes interpretation of the first part of the curve unreliable, the mean cell life is in practice often the least valid deduction that can be made from a 10-day study.

Alternatively, it would be possible to express the results in terms of half-life of ^{51}Cr in the circulation uncorrected for elution. To this approach may be offered two objections. First, in slow regressions, determination of the half-life point may require considerable extrapolation. Secondly, this use of the concept of half-life is meaningful only if the regression is strictly exponential to the point of near extinction. As Dornhorst (1951) showed, this is true only of red blood cell populations subject to random destruction so intense that age-loss can virtually be ignored.

To meet the objections given above, and yet keep the study short, we have adopted the following method of interpretation. Total ^{51}Cr loss is treated as a single exponential function over the period 48 hours to 10 days, and the rate of loss (percentage loss ^{51}Cr per day) is determined. For normal subjects it has been shown that if the ^{51}Cr survival is plotted in this way the departure from linearity, if the period of "early loss" (0-48 hours) is excluded, is slight over the first 50 days (Mollison and Veall, 1955). Certainly over the short period we have chosen the points are well fitted by a straight line on semi-logarithmic paper. Normal curves fall so slowly that, even were they linear, they would not materially deviate from an exponential over this portion of the curve, whereas rapidly falling curves are likely to represent random destruction and will therefore approximate to exponentials. Thus in each case a measure of rate of loss can be obtained using a 10-day curve. This use of the data facilitates comparison of rates of ^{51}Cr loss in the same subject over two or more short periods of observation, e.g., before and after treatment.

In certain haemolytic syndromes there is evidence that early ^{51}Cr elution may be decreased and this may mask shortened red blood cell life (Mollison, 1959; Jones, N. F., unpublished). It is thus not legitimate to fix an upper limit for the normal rate of ^{51}Cr loss over a period as short as 10 days. We therefore approached this problem empirically. In 10 normal subjects, from whom samples were obtained in the standard sequence over days 2 to 10, ^{51}Cr loss did not exceed 4.25% per day. In

patients with undoubted haemolysis shown by conventional haematological methods, we have observed over the same period rates of ^{51}Cr loss varying from 5.5% to 19% per day. On the basis of this experience it appeared that, for this disposition of blood samples, rates of ^{51}Cr loss greater than 5.0% per day could be regarded as abnormal.

An analysis of our results in the group of normal subjects and an investigation of the experimental error showed that only one in 20 normal subjects would be expected to show rates of ^{51}Cr loss exceeding 5.1% per day if the samples were taken in our standard sequence. As this figure agreed closely with the 5.0% per day arrived at empirically, we have taken 5.1% per day to be the critical value above which rates of ^{51}Cr loss are abnormal. It must be recognized that a rate of ^{51}Cr loss of less than 5.1% per day over this short period does not necessarily imply normal red blood cell survival; but it may then be inferred that haemolysis is not contributing greatly to the patient's anaemia. With so short a study it is not permissible to calculate mean cell life, and when this is suspected of being only

slightly shortened the period of ^{51}Cr study must be extended.

While, therefore, this 10-day technique has obvious limitations, it is extremely valuable both in clinical laboratory practice and for assessing the effects of treatment on red cell survival in rapidly changing haematological states. It may also be combined with a ^{59}Fe study of red cell production.

We wish to thank the consultant physicians and surgeons of St. Thomas's Hospital for permission to study their patients, Professor A. C. Dornhorst for his advice and criticism, and Dr. P. Armitage, of the Medical Research Council's Statistical Research Unit, London School of Hygiene and Tropical Medicine, for his advice on the statistical aspects of this investigation. The expenses of the investigation were partly borne by the Endowment Fund, St. Thomas's Hospital.

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