An Evaluation of DFP$^{32}$ and Cr$^{51}$ as Methods of Measuring Red Cell Life Span in Man

By Martin J. Cline and Nathaniel I. Berlin

Gray and Sterling's method of labeling red cells with chromium$^{51}$ provided the first radioisotopic method for measurement of the red cell life span with in vitro labeled cells. Because of the ready availability of Cr$^{51}$, the ease of red cell labeling and of quantitative isotope measurement, the Cr$^{51}$ method came into widespread use. The most common method of analysis of Cr$^{51}$ red cell survival data is to plot the logarithm of the Cr$^{51}$ content per ml. RBC as a function of time and to fit the data for the first 30 to 40 days to a straight line and derive a half-time. Other methods of analysis of the Cr$^{51}$ red cell survival data are possible, but are not often used because of their complexity or time requirement.

The only serious challenge to the use of Cr$^{51}$ as a clinical tool for measuring the red cell life span was the suggestion of Grob et al. that the cholinesterase inhibitor, diisopropylfluorophosphate (DFP), might be used to measure erythrocyte survival in man. This, Cohen and Warringa did with DFP labeled with P$^{32}$. Since Cohen and Warringa's original report, DFP$^{32}$ has been used to measure erythrocyte survival in several species of experimental animals and in over 70 humans.

The present study was undertaken to compare the DFP$^{32}$ and Cr$^{51}$ technics as clinical tools in the measurement of erythrocyte life span. The C$^{14}$-glycine technic was used as a standard of reference for comparison with the other methods.

Patients and Methods

The red cell life span was measured with C$^{14}$-labeled glycine in 39 patients with hematologic and/or malignant disease. In 20 of these patients, the red cell life span was determined with DFP$^{32}$, and in 33 patients the Cr$^{51}$ T$^{1/2}$ was determined.

Red cell life span curves were obtained following the intravenous administration of 100 μc. of glycine-2-C$^{14}$ by methods previously described. The life span of the red cells by the C$^{14}$-glycine method was taken as the time between one-half the maximal plateau value on the ascending and descending limbs of the curve of specific activity versus time when the life span was finite. When there was marked random destruction of erythrocytes, the life span was defined as the inverse of the fraction destroyed per day.

The apparent red cell survival half-time (T$^{1/2}$) was determined with radiochromium by the method of Gray and Sterling as modified by Read. The first part of the red cell Cr$^{51}$ curve was visually fitted to a straight line on semi-logarithmic paper with time in days plotted on the abscissa, and counts per minute per ml. of red cells on the ordinate. The half-time was estimated from the period between day 1 and day 35. This method of analysis of the Cr$^{51}$ data was chosen because it is the one most often employed. In normal individuals, the observed Cr$^{51}$ T$^{1/2}$ from different laboratories ranges from 25 to 34 days.

From the Metabolism Service, National Cancer Institute, National Institutes of Health, Bethesda, Md.

Submitted Apr. 23, 1962; accepted for publication Apr. 20, 1963.

BLOOD, Vol. 22, No. 4 (October), 1963
DFP32 (specific activity 80–220 μc./mg.)* obtained monthly in sterile propylene glycol was administered intravenously in a single dose of 0.5–0.9 mg. Venous blood samples were obtained 3 times the 1st week and at approximately weekly intervals thereafter for 25–70 days. The plasma was separated and the red cells were washed 3 times in cold isotonic saline to remove the remaining plasma, white blood cells, and platelets, after which an approximately equal volume of saline was added. The hematocrit of the red cell suspension was determined in quintuplicate and 1 ml. aliquots of the washed red cells were pipetted in duplicate into disposable boats of aluminum foil and dried at 70°C. The boats were then sealed in cellophane wrapped around a Geiger-Muller tube and counted. Maximum counting error was 3 per cent. The counts per minute per ml. of red blood cells due to DFP32 were computed from the radioactivity of the red cell suspension and its hematocrit. Where the temporal decline in specific activity was linear, the data were fitted to a straight line by the method of least squares. The mean red cell life span was obtained by extrapolation of this line to the abscissa. When there was random destruction of erythrocytes, the life span was defined as the inverse of the fraction destroyed per day.

**RESULTS**

Experimental Results

**DFP32 and C14-glycine:** The data from 20 patients with simultaneous DFP32 and C14-glycine red cell survival studies are shown in table 1. All the patients were in steady state conditions at the time of the study. There was good agreement between the life spans obtained by the C14-labeled glycine and DFP32 technic as shown in figure 1.

**Cr51 and C14-glycine:** Simultaneous erythrocyte C14-glycine life spans and Cr51 half-times (T½) are plotted in figure 2. The red cell survivals in those patients who received only labeled glycine and Cr51 are plotted as closed circles. The erythrocyte life spans in those patients who also received DFP32 are shown as open circles. The broken lines represent a red cell life span of 100 days by the C14-labeled glycine technic and a Cr51 T½ of 25 days. Below the area enclosed by the solid lines are eight cases where the red cell life span was short by the glycine technic but which have Cr51 half-times greater than 25 days. It is apparent that in these eight cases, the Cr51 method failed to reveal a shortening of red cell survival.

**DISCUSSION**

The red cell life spans obtained with DFP32 agrees very well with those obtained with labeled glycine. From this it is concluded that DFP32 is a reliable tool for measuring erythrocyte life span.

Several investigators have reported a transient rapid decrease in red cell P32 content during the first few days after DFP32 administration.6,8,16,17 This loss of label appears to be in part dependent on the dose of administered DFP32 and has been ascribed to elution from intact surviving cells.16,17 In the doses of DFP32 used in this study, this early loss of label was observed in only three patients. In each of these three cases, this rapid early loss was completed by the 3rd day after isotope administration. In these three patients, the differences between the observed specific activity on day 1 following isotope administration and the specific activity calculated from the least

*New England Nuclear Corp.*
DFP\textsuperscript{32} AND CR\textsuperscript{51} IN MEASURING RBC LIFE SPAN

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease</th>
<th>( \text{C}\textsuperscript{14}-\text{glycine Life Span} ) (days)</th>
<th>( \text{DFP}\textsuperscript{32} ) Life Span (days)</th>
<th>( \text{Cr}\textsuperscript{51} ) T\textsubscript{1/2} (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>L. O.</td>
<td>Multiple myeloma</td>
<td>67</td>
<td>69</td>
</tr>
<tr>
<td>2.</td>
<td>H. W.</td>
<td>Multiple myeloma</td>
<td>84</td>
<td>76</td>
</tr>
<tr>
<td>3.</td>
<td>R. D.</td>
<td>Multiple myeloma</td>
<td>90</td>
<td>84</td>
</tr>
<tr>
<td>4.</td>
<td>M. S.</td>
<td>Multiple myeloma</td>
<td>99</td>
<td>97</td>
</tr>
<tr>
<td>5.</td>
<td>H. P.</td>
<td>Multiple myeloma</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>6.</td>
<td>M. S.</td>
<td>Chronic myelocytic leukemia</td>
<td>120</td>
<td>118</td>
</tr>
<tr>
<td>7.</td>
<td>W. B.</td>
<td>Chronic myelocytic leukemia</td>
<td>72</td>
<td>54</td>
</tr>
<tr>
<td>8.</td>
<td>M. G.</td>
<td>Waldenström's macroglobulinemia</td>
<td>55*</td>
<td>52*</td>
</tr>
<tr>
<td>9.</td>
<td>A. S.</td>
<td>Waldenström's macroglobulinemia</td>
<td>110</td>
<td>93</td>
</tr>
<tr>
<td>10.</td>
<td>H. R.</td>
<td>Waldenström's macroglobulinemia</td>
<td>93</td>
<td>81</td>
</tr>
<tr>
<td>11.</td>
<td>B. C.</td>
<td>Waldenström's macroglobulinemia</td>
<td>63</td>
<td>72</td>
</tr>
<tr>
<td>12.</td>
<td>H. G.</td>
<td>Waldenström's macroglobulinemia</td>
<td>40*</td>
<td>50*</td>
</tr>
<tr>
<td>13.</td>
<td>M. T.</td>
<td>Waldenström's macroglobulinemia</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>14.</td>
<td>M. B.</td>
<td>Macroglobulinemia and reticulum</td>
<td>57</td>
<td>55</td>
</tr>
<tr>
<td>15.</td>
<td>M. D.</td>
<td>Hodgkin's disease</td>
<td>61</td>
<td>68</td>
</tr>
<tr>
<td>16.</td>
<td>W. B.</td>
<td>Hodgkin's disease</td>
<td>65</td>
<td>64</td>
</tr>
<tr>
<td>17.</td>
<td>H. K.</td>
<td>Chronic lymphatic leukemia</td>
<td>91</td>
<td>94</td>
</tr>
<tr>
<td>18.</td>
<td>D. R.</td>
<td>Chronic lymphatic leukemia</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>19.</td>
<td>L. O'N.</td>
<td>Acute lymphatic leukemia</td>
<td>27*</td>
<td>34*</td>
</tr>
<tr>
<td>20.</td>
<td>H. B.</td>
<td>Lymphosarcoma</td>
<td>102</td>
<td>102</td>
</tr>
</tbody>
</table>

\*Calculated from an exponential decrease in red cell specific activity.

Squares fitted line established subsequent to day 1 ranged from 3.8 per cent to 7.4 per cent. Thus no more than 7.4 per cent of the dose was eluted from intact surviving cells after day 1.

Like all other existing methods of measuring the erythrocyte life span, the DFP\textsuperscript{32} technic has certain limitations. As usually utilized, the methods which involve random labeling of a population of red cells heterogeneous with respect to age (DFP\textsuperscript{32}, Cr\textsuperscript{51} and the Ashby technic) depend for the validity of the answers they produce on the maintenance of a constant total red cell volume. When there is an inadequate rate of erythropoiesis resulting in a falling total red cell volume, there will be failure of dilution of labeled cells by newly synthesized unlabeled erythrocytes. Under these circumstances the rate of change of red cell specific activity will be unusually slow and the calculated red cell life span will be falsely long. Conversely, when there is an expanding total red cell volume, there will be an increased rate of dilution of labeled red cells by unlabeled cells with the result that the calculated red cell life span will be falsely short. The C\textsuperscript{14}-glycine method which labels a group of cells of similar age by biosynthetic incorporation of the isotope into hemoglobin is less sensitive to changes in total red cell volume, although here, too, data obtained during non-steady state conditions must be interpreted with caution. In all methods, if serial measurements of the total red cell volume are made, the total circulating isotope can be calculated and from this the red cell life span correctly determined.

In contrast to the DFP\textsuperscript{32} method, the data presented here show that the
Cr$^{51}$ technic does not compare favorably with the labeled glycine method if 25 days is taken as the lower limit of normal. If, however, 27 or 28 days is taken as the normal lower limit, then only rarely does the Cr$^{51}$ survival fail to reflect shortening of red cell life span. Invariably, when the Cr$^{51}$ survival was less than 25 days, the red cell life span was less than 80 days.

Some degree of failure of agreement between the Cr$^{51}$ and labeled glycine technic is to be anticipated in some cases. It must be recalled that the Cr$^{51}$ half-time is a compromise in which the data are forced to fit to a single exponential curve. The decrease in red cell Cr$^{51}$ specific activity is under normal circumstances a function of two variables: the linear decrease in radioactivity due to replacement of senescent cells by newly synthesized erythrocytes and the exponential rate of elution of Cr$^{51}$ from intact erythrocytes. Evidence has been obtained in this laboratory that the range of Cr$^{51}$ elution rates observed in disease may be quite wide, from 0.62 per cent to 2.27 per cent per day.\textsuperscript{15} In addition, in disease states there may be random loss of red cells due to hemolysis and/or blood loss which may be wholly or partly exponential in character.

The use of DFP$^{32}$ as a red cell label eliminates the variable of continual elution from intact surviving cells. When red cell life span is finite, the decrease in red cell radioactivity is linear. When random erythrocyte destruction predominates, the specific activity decreases in an exponential fashion. Data analysis therefore is considerably easier with DFP$^{32}$ than with Cr$^{51}$. 

---

**Fig. 1.**—The red cell life span determined simultaneously with DFP$^{32}$ and C$^{14}$-glycine.
DFP$^{12}$ AND CR$^{51}$ IN MEASURING BLOOD LIFE SPAN

**Fig. 2.**—The red cell life span determined with C$^{14}$-glycine compared to red cell Cr$^{51}$ T$^{1/2}$. Lines indicate lower limit of normal for each method—100 days for C$^{14}$-glycine, and 25 days for Cr$^{51}$ T$^{1/2}$; open circles—patients also studied with DFP$^{32}$.

DFP$^{32}$ compares favorably with Cr$^{51}$ in the ease of sample preparation for counting. In addition, the tagging of red cells with DFP$^{32}$ is considerably easier than with Cr$^{51}$, requiring only a single intravenous injection. DFP$^{32}$ has the disadvantage of greater cost and somewhat greater estimated radiation dose.\(^{10}\)

**SUMMARY**

DFP$^{32}$ or Cr$^{51}$ red cell survival studies were performed in 39 patients with hematologic disorders. In each case the erythrocyte life span was also determined using the C$^{14}$-labeled glycine technic.

Good agreement was found between the survival data obtained by the DFP$^{32}$ and the glycine method. The red cell Cr$^{51}$ T$^{1/2}$ failed to reflect moderate shortening of red cell survival in a significant number of cases when a 25 day half-time was taken as the lower limit of normal. The occasional discrepancy between the erythrocyte life spans obtained with labeled glycine and the Cr$^{51}$ halftime is probably the result of the limitations of the prevalent method of Cr$^{51}$ data analysis and of the wide range of Cr$^{51}$ elution rates in disease.

Because of its simplicity and reliability, the DFP$^{32}$ technics appears to be the method of choice for the determination of red cell survival.

**SUMMARIO IN INTERLINGUA**

Diisopropylfluorophosphata a phosphoro radioactive (DFP$^{32}$) e chromo radioactive (Cr$^{51}$) eseva usate in studios del longevitate erythrocytic in 39
patients con disorders hematologic. In omne caso le longevitate erythrocytic eseva etiam determinate per medio del methodo a glycina marcate con C\textsuperscript{14}.

Un bon accordo eseva constatate inter le datos de longevitate obtenite per le methodo a DFP e le methodo a glycina. Le tempore de medie valor pro le longevitate erythrocytic obtenite con Cr\textsuperscript{51} non reflecteva moderate reductiones del longevitate erythrocytic in un numero significative de casos quando un tempore de medie valor de 25 dies esseva acceptate como limite inferior del norma. Le discrepantia occasional inter le longevitates obtenite pro le erythrocytos a base del tempore de medie valor de marcate glycina e de Cr\textsuperscript{51} es probablemente le resultato del prevalente methodo de analyser datos a Cr\textsuperscript{51} e del extense campo de variationes in le intensitates del elution de Cr\textsuperscript{51} in statos pathologic.

A causa del simplicitate e fidelitates del technicas a DFP\textsuperscript{32}, istos pare esser le methodo de election pro le determination del longevitate erythrocytic.

REFERENCES

12. Eadie, G. S., Smith, W. W., and Brown, I. W., Jr.: The use of DFP\textsuperscript{32} as a red cell tag with and without simultaneous tagging with chromium\textsuperscript{51} in certain animals in the presence or absence of random destruction. J. Gen. Physiol. 43:825, 1960.
and turnover using radiochromium.
15. Mollison, P. L.: Further observations on
the normal survival curve of 51Cr-
labelled red cells. Clin. Sc. 21:21,
1961.
16. Hjort, P. F., Paputchis, H., and Cheney,
B.: Labeling of red blood cells with
radioactive diisopropylfluorophosphate
(DFP<sup>32</sup>): Evidence for an initial re-
17. Cline, M. J., and Berlin, N. I.: Simultaneous measurement of the survival
of two populations of erythrocytes
with the use of labeled diisopropyl-
18. —, and —: The red cell chromium elu-

Martin J. Cline, M.D., formerly Clinical Associate, Metabolism
Service, National Cancer Institute, Bethesda, Md. Present ad-
dress: Helen Hay Whitney Foundation Fellow, Department of
Medicine, University of Utah, Salt Lake City, Utah.

Nathaniel Berlin, M.D., Ph.D., Clinical Director, National
Cancer Institute, Bethesda, Md.
An Evaluation of DFP$^{32}$ and Cr$^{51}$ as Methods of Measuring Red Cell Life Span in Man

MARTIN J. CLINE and NATHANIEL I. BERLIN