The Life Span of Red Cells in the Rat and the Mouse as Determined by Labeling with DFP\(^{32}\) in Vivo

By L. M. van Putten

*With the technical assistance of Fineke Croon*

Several methods have been used to determine the life span of rat erythrocytes. Ponticorvo\(^1\) determined the deuterium content of hemin after feeding this nuclide to rats and estimated the survival at 100 days. Berlin, Huff and Hennessy\(^2\) arrived at the same estimation by determining the turnover of Fe\(^{59}\) in hemoglobin. In the same laboratory, studies with glycine-C\(^{14}\)-tagged hemoglobin showed a mean survival of 68 days.\(^3\) Finch and co-workers determined the red cell life span after transfusions of Fe\(^{59}\)-tagged cells and found it to be from 45 to 50 days.\(^4\) With the aid of Cr\(^{51}\) they estimated the average survival time at 50 days.\(^5\) Their work is based on the assumption that red cell destruction in the rat is a random phenomenon, an assumption at variance with the observations of Berlin.\(^3\)

Few recent data on the life span of mouse red cells were found in the literature. Dod, Bierman and Shimkin\(^6\) determined the life span from the disappearance of sulfhemoglobin from the red cells and found a maximal survival of about 19 days. Burnell, Brickley and Finch\(^4\) injected Fe\(^{59}\)-tagged red cells and followed the disappearance of radioactivity from the blood. They calculated an average survival time of 20 to 30 days.

The use of diisopropylphosphorofluoridate tagged with radioactive phosphorus (DFP\(^{32}\)) as a labeling agent for human blood cells was first introduced by Cohen and Warringa\(^7\) in 1954, and its successful use has been reported by several investigators.\(^8\)\(^-\)\(^13\) Because of the simplicity of this method of labeling cells in vivo, its applicability for small laboratory animals was investigated.

**Materials and Methods**

The experimental animals were male W.A.G. rats (an inbred Wistar Albino strain, obtained in 1953 from the Glaxo laboratories) weighing 235-250 Gm. and male F1 mice (CBA x C57BL) weighing 22-27 Gms. DFP\(^{32}\) was obtained initially from the Medical Biological Laboratory of the National Defense Research Council where it was synthesized by Dr. R. Oosterbaan. Later it was synthesized by Dr. H. M. Klouwen in our Institute. DFP\(^{32}\) with a specific activity between 20 and 200 \(\mu\text{C}./\text{mg}\). DFP was dissolved in peanut oil and administered by intramuscular injection. The resultant radioactivity in the red cells was determined by the method of Cohen et al.\(^7\) Red cells from heparinized blood were washed 3 times in at least 5 volumes of saline and hemolyzed in distilled water. The volume of the hemolysate was made up to 10 ml, and the radioactivity compared in a liquid C.M. counter with that of a standard, obtained by hydrolyzing a known amount of DFP\(^{32}\). At least 4000 counts from each sample were registered. Total nitrogen was determined in a portion of each hemolysate.

From the Radiobiological Institute of the National Health Research Council, TNO, Rijswijk, The Netherlands.

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The activity of the blood samples was expressed in micrograms DFP per gram erythrocyte-nitrogen. (One gram of nitrogen is contained in about 4x10⁹ rat erythrocytes or 5x10⁹ mouse erythrocytes.) In a preliminary experiment it was found that the extent of labeling of red cells in the rat was independent of the dosage between 30 and 100 μg. DFP per rat.

**The Life Span of Rat Erythrocytes**

Forty μg. DFP was administered to each of 40 rats. At various times after the injection, groups of five rats were killed and the radioactivity in the red cells was determined. The results are presented in table 1 and figure 1. In the first week, the amount of P in the red cells showed a sharp decrease. After the eighth day, however, the loss of radioactivity was much slower. The early steep fall cannot be due to red cell destruction (T₁/₂ < 1 day; the red cell counts in the peripheral blood of the rats did not vary between day 1 and day 4). It can only reflect loss of part of the activity from the red cells. The slow decline after the eighth day is linear with time. As it is highly improbable that this could represent elution of the label from the cells, it is assumed that this part of the curve reflects the disappearance of labeled red cells from the circulation. The regression formula for the data after day 5, as calculated by the method of least squares, is Y = 1.095 - 0.01825 X, and the life span of the red cells is 60 ± 3.2 (S.D.) days.

**The Life Span of Mouse Erythrocytes**

Fifteen μg. DFP was administered to each of 30 mice. At various times after injection, groups of 5 mice were killed and the radioactivity in the red cells was determined. The results are given in table 2 and figure 2. The disappearance curve is linear with time and there is no evidence of elution or random destruction of erythrocytes. The regression formula calculated by the method of least squares is Y = 3.706 - 0.0911 X, and the red cell life span is 40.7 ± 1.9 (S.D.) days. In a second experiment with mice, a method of determining the red cell life span in individual animals was investigated. Fifteen μg. of DFP was administered to each of 10 mice. At 5 and at 18 days after this injection the animals were bled to determine the radioactivity in the red cells. The amount of blood withdrawn at 5 days did not exceed 0.05 ml., which is estimated to correspond to 3 per cent of the total blood volume. From the decline in radioactivity the life span of the red cells was calculated

| Table 1.—Phosphorus Found in Erythrocytes of Rats After Injection of 40 μg. DFP |
|-----------------|---------------------------------|
| Days after injection | μg. DFP/Gm. erythrocyte-nitrogen |
| 1               | 2.18 ± 0.10                     |
| 4               | 1.30 ± 0.03                     |
| 8               | 0.94 ± 0.03                     |
| 15              | 0.80 ± 0.03                     |
| 22              | 0.70 ± 0.03                     |
| 29              | 0.55 ± 0.03                     |
| 36              | 0.48 ± 0.01                     |
| 43              | 0.31 ± 0.01                     |

Each figure expresses the mean value (±S.E.) for 5 rats.
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\[ Y = 1.095 - 0.001825 X \]
\[ L = 60 \text{ days} \]

Fig. 1.—Amount of P\(^\text{32}\) found in rat erythrocytes after injection of DFP\(^\text{32}\) (expressed as \(\mu\text{g. DFP/Gm. erythrocyte-nitrogen}\)). The vertical bars give the standard errors of the mean for each group of 5 rats. The straight line has been calculated according to the method of least squares from the data after the fifth day.

for each animal. The resulting values were: 28, 41, 41, 42, 44, 45, 50, 54 and 59 days; mean and S.D. were 44.6 ± 8.1.

DISCUSSION

The finding of an elution of the label from rat erythrocytes deserves comment because this phenomenon has not been reported before with DFP as a labeling agent. A preliminary experiment in the rat, using a dose of 100 \(\mu\text{g. DFP}\), resulted in a similar tagging of the red cells as reported above: the total amount found in the red cells after 1 day was 2.07 \(\mu\text{g.}/\text{Gm. nitrogen}\), and the amount of label which was not subject to elution was extrapolated at 1.11 \(\mu\text{g.}/\text{Gm. nitrogen}\). In the experiment with 40 \(\mu\text{g. DFP}\) per rat these values were 2.18 and 1.095 \(\mu\text{g.}/\text{Gm. nitrogen}\), respectively.

<table>
<thead>
<tr>
<th>Days after injection</th>
<th>(\mu\text{g. DFP/Gm. erythrocyte-nitrogen})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.72 ± 0.06</td>
</tr>
<tr>
<td>5</td>
<td>3.23 ± 0.06</td>
</tr>
<tr>
<td>8</td>
<td>2.95 ± 0.09</td>
</tr>
<tr>
<td>11</td>
<td>2.59 ± 0.06</td>
</tr>
<tr>
<td>21</td>
<td>1.85 ± 0.03</td>
</tr>
<tr>
<td>28</td>
<td>1.16 ± 0.05</td>
</tr>
</tbody>
</table>

Each figure expresses the mean value (±S.E.) for 5 mice.
Fig. 2.—Amount of $^{32}$P found in mouse erythrocytes after injection of DFP$^{32}$ (expressed as $\mu$g. DFP/Gm. erythrocyte-nitrogen). The vertical bars give the standard errors of the mean for each group of 5 mice.

The close similarity in the extent of labeling of rat erythrocytes in the two experiments covering rather different dosages suggests that a saturation has occurred for both mechanisms by which DFP is bound by the cell, a stable combination and a labile one which is reversible in vivo. It has been calculated that each rat erythrocyte binds irreversibly ± 9000 molecules of DFP, a number which is lower than that found in the mouse (at least 23,000) and in man (at least 12,500). (In man the dosage used for labeling red cells and thrombocytes has been much lower than 0.1 mg./Kg., the dose at which saturation of rat erythrocytes is just approached. It is thus possible that human red cells are able to bind an appreciably higher amount of DFP.) About the actual sites of binding of DFP in rat erythrocytes, no definite data are available.

The linearity of the second part of the disappearance curve for the erythrocytes of the rat conforms to the existence of a definite life span and bears evidence against random destruction of cells. The calculated life span is in reasonable agreement with the more recent values in the literature.$^{3,5}$

In the case of the mouse the experimental results are strongly suggestive of a true life span without random destruction.
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The linearity of the normal disappearance curve may be of great advantage for determinations of the life span of red cells in pathologic conditions in this species. The finding of random destruction by earlier workers is probably due to the use of less suitable tagging methods. It seems unlikely that it would be due to the use of different mouse strains.

SUMMARY AND CONCLUSIONS

The red cells of rats and mice were tagged in vivo by injection of diisopropylphosphorofluoridate (DFP), labeled with $^{32}$P, and the disappearance of radioactivity from the circulating red cells was determined. From the data obtained, it is concluded that the disappearance of the red cells is linear with time and that the red cells in both rats and mice have a true life span without measurable random destruction.

The life span of the erythrocytes was found to be $60.0 \pm 3.2$ (S.D.) days in the rat and $40.7 \pm 1.9$ (S.D.) days in the mouse.

Especially in the mouse the determination of the red cell life span with DFP$^{32}$ is a simple procedure.

SUMMARIO IN INTERLINGUA

Le erythrocytos de rattos e muses esseva marcate in vivo per le injection de di-isopropylphosphorofluoridato a $^{32}$P, e le disparition del radioactivitate ab le erythrocytos circulante esseva determinate. Super le base del resultatos obtenite, le conclusion es formulata que le disparition del erythrocytos es linear con le tempore e que le erythrocytos tanto in rattos como etiam in muses ha un ver duration vital sin mesurabile destruction al hasardo. Le duration vital del erythrocytos esseva 60,0 dies in le ratto e 40,7 dies in le mus con deviaziones standard de $\pm 3,2$ e $\pm 1,9$ dies, respectivemente.

Le determination del duration vital de erythrocytos per medio de iste methodo—specialmente in muses—es un simple manovra.

REFERENCES


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