Brief Report

The Early Loss of Radioactivity in Cr\textsuperscript{51} Survival Curves:
Destruction of Cells or Loss of the Label?

By N. Kleine and H. Heimpele

The tagging of red cells with radioactive sodium chromate, introduced in 1950 by Gray and Sterling,\textsuperscript{1} is widely used for determination of red cell volume and red cell life span. Difficulties in the interpretation of survival studies with Cr\textsuperscript{51} arise from the well-known elution of the label and the additional loss of specific red cell activity within the first 24 hours after the injection of the labeled red cells. The mechanism of this early loss of radioactivity is not fully understood. Some authors claim a destruction of cells damaged by the collection and handling in vitro, while others believe that a rapid elution of a small percentage of the radioactive label takes place. To solve this problem we tagged human erythrocytes simultaneously with DFP\textsuperscript{32} and Cr\textsuperscript{51} and followed the specific red cell activities within the first 24 hours after transfusion of the labeled cells. The results point to a rapid elution as the cause of this early loss observed in radiochromium survival curves.

Methods

When red cells are labeled with DFP\textsuperscript{32}, some of the label is nonspecifically bound and rapidly eluted,\textsuperscript{2,3} particularly when the specific binding capacity of red cells is surpassed by tagging in vitro. We therefore tagged the erythrocytes of a donor in vivo. His red cells were transfused 48 hours later after additional labeling with Cr\textsuperscript{51} in vitro. After this period all the DFP\textsuperscript{32} is irreversibly bound to the esterases. The details of the procedure were as follows: 150 \(\mu\)c. of DFP\textsuperscript{32} (specific activity, 300 \(\mu\)c./mg.) in propylene glycol was diluted with 10 ml. of normal saline and injected intravenously into the donor. The injection was extended over 10 minutes to ensure an optimal incorporation rate.\textsuperscript{4} Forty-eight hours later 400 ml. of blood was drawn into a plastic bag containing an appropriate amount of heparin or ACD-solution. After 24 hours storage at 4 C., 100 \(\mu\)c. Cr\textsuperscript{51} was added to the bag with gentle agitation. The bag was kept 45 minutes at room temperature and 50 mg. ascorbic acid was added to prevent further labeling. The blood was then given to a compatible healthy recipient within 60 minutes. Samples were taken from the bag and from the recipient at 10 minutes, 30 minutes and 24 hours after the end of the transfusion.

Microhematocrit and cyanhemoglobin determinations were made from all the samples. One ml. of whole blood was pipetted into metal planchets containing exactly fitted discs of filter paper and dried by air. Preparations of 1 ml. of plasma were made in the same way. The gamma-emission of Cr\textsuperscript{51} was measured with a scintillation counter, the bremsstrahlen of P\textsuperscript{32} being absorbed by 1 cm. of plastic material. The high energy \(\beta\)-rays of P\textsuperscript{32} were counted with an end-window G-M-tube (low background counter). Fifteen to 21 per cent of the Cr\textsuperscript{51} emission was recorded in the G-M-tube and subtracted from the P\textsuperscript{32} counts.
All determinations were made in duplicate. The statistical error of radioactive counting was maximally 3 per cent.

RESULTS

No significant difference was observed between red cell radioactivity of 10 and 30 minute samples (table 1). This result has to be expected in view of the relatively long period of transfusion itself. When the average of both values is taken as 100 per cent, the difference of P³² and Cr⁵¹ red cell specific activity after 24 hours is more significant (table 2).

The decrease of P³² activity agrees closely with the expected values assuming a mean red cell life span of 115 days in the absence of random destruction. It provides no evidence of early destruction of a measurable quantity of the transfused cells. The additional loss of Cr⁵¹ radioactivity must be attributed to a rapid elution of this label. The magnitude of the early loss in ACD-preserved blood is comparable to the reported values; in heparin-preserved blood it is distinctly greater. This accelerated early elution from heparin-preserved cells could be confirmed in additional transfusions labeled with Cr⁵¹ alone.

DISCUSSION

When red cells are labeled with Cr⁵¹ in vitro and reinjected, the specific activity of the erythrocytes declines in an approximately exponential fashion with a biologic half-life of 25 to 35 days. There is general agreement that an additional early loss of radiochromium activity occurs within the first 24 hours, which seems to be slightly smaller if large volumes of cells are labeled in vitro or if the whole red cell mass is labeled in vivo. The mechanism of this early loss, however, is still a matter of discussion. It cannot be explained by a normal population of short lived erythrocytes, because this is quantitatively in no way compatible with the well established values of a normal red cell turnover rate. An actual loss of a small proportion of cells due to the collection and handling procedure was accepted by Hughes-Jones and Mollison, Donohue et al., Strumia et al., Borel et al. and Emerson and Bove, who measured directly the specific activity of Cr⁵¹ tagged donor cells.

Table 1

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<tr>
<th></th>
<th>ACD</th>
<th>Heparin</th>
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<tbody>
<tr>
<td></td>
<td>24 hr./10 min.</td>
<td>24 hr./30 min.</td>
</tr>
<tr>
<td>No. of observations</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>P³²</td>
<td>1.005 ± 0.026</td>
<td>0.980 ± 0.042</td>
</tr>
<tr>
<td>Cr⁵¹</td>
<td>0.953 ± 0.015</td>
<td>0.953 ± 0.012</td>
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Table 2

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<thead>
<tr>
<th></th>
<th>ACD</th>
<th>Heparin</th>
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<tbody>
<tr>
<td></td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>P³²</td>
<td>(99.4 ± 2.5)%</td>
<td>(98.6 ± 1.4)%</td>
</tr>
<tr>
<td>Cr⁵¹</td>
<td>(95.3 ± 1.3)%</td>
<td>(83.4 ± 1.5)%</td>
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recovered from the recipient by the technic of differential agglutination.

In more recent publications, Mollison and his coworkers held a rapid component of Cr\textsuperscript{51} elution responsible for the early loss\textsuperscript{11,12}. This assumption was mainly based on the small but significant early loss observed after labeling of the cells in vivo by intravenous injection of Cr\textsuperscript{51}. Attempts to solve the problem by simultaneous observation of transfused cells with Cr\textsuperscript{51} and the Ashby method have failed because of the relative inaccuracy of the latter technic within the first few days after transfusion\textsuperscript{12}. Our results with DFP\textsuperscript{32} tagged erythrocytes show a normal survival of cells after 24 hours of storage; this is in accordance with the few published observations on the fate of transfused red cells after short-time preservation\textsuperscript{7,13,14}. The additional early loss of radiochromium activity must be attributed to an elution of a small part of the label, because there is no evidence suggesting a difference in the amount of labeling with Cr\textsuperscript{51} within the red cell populations of normal blood, as demonstrated by Kleine\textsuperscript{15}. The higher rate of elution from heparinized cells points to differences according to the anticoagulants used and may be explained by the different binding of chromate to the intracellular substances as a function of pH. With lower pH the relative amount of Cr\textsuperscript{51} attached to the hemoglobin is increased\textsuperscript{15}. Similar differences were observed by Mollison\textsuperscript{11} with citrate solutions of varying pH-values. The 24 hour "survival" of radiochromium labeled red cells is widely used as an index of viability after collection and storage of blood under varying conditions. Our findings show that the elution of Cr\textsuperscript{51} during this period must be considered in the interpretation of the results of such studies. In particular it should be taken in consideration that the rate of elution is not constant, as could be shown by the results obtained with heparinized and ACD blood.

**Summary**

Using human erythrocytes labeled simultaneously with DFP\textsuperscript{32} and Cr\textsuperscript{51}, studies of specific red cell activities within the first 24 hours after infusion of labeled cells were carried out. The results obtained suggest that the early loss of Cr\textsuperscript{51} specific red cell activity which has been observed in radiochromium survival curves is due to rapid elution of a portion of the label.

**Summary in Interlingua**

Con le uso de erythrocytos human marcate simultaneamente con diisopropyl-fluorophosphato a phosphoro 32 e con Cr\textsuperscript{51}, studios de specific activitates erythrocytic intra le prime 24 horas post le infusion de marcate cellulas eseva effectuate. Le resultatos obtenite indica que le precoce perdita de activitate erythrocytic a Cr\textsuperscript{51} que ha essite observe in curvas de longevitate es probablemente cause per un rapide elution de un portion del marca.

**REFERENCES**


2. Hjort, P. F., Paputchis, H., and Cheney, D.: Labelling of red blood cells with radioactive diisopropylfluorophosphate (DFP\textsuperscript{32}), evidence for a initial re-


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