Red Cell Preservation: Further Studies with Adenine

By Ernest R. Simon

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In a previous communication we have shown that supplementing the acid citrate dextrose (ACD) preservative with small amounts of adenine (0.75 ± 0.25 μM per ml. ACD-blood) at the beginning of the storage period preserves satisfactory viability (post-transfusion survival greater than 70 per cent) of stored human erythrocytes for at least 5 weeks. Larger amounts of adenine were less effective. The improved viability was associated with higher intracellular adenosine triphosphate (ATP) levels throughout storage and better preservation of erythrocyte glycolytic capacity, measured at 37 C. at the end of the storage period. Despite the maintenance of higher ATP levels with adenine, glucose consumption and lactate formation throughout the storage period were not appreciably different from the ACD controls. The simultaneous addition of relatively large amounts of inosine (10 μM per ml. ACD-blood) had little further effect in extending viability.

The purpose of the present communication is to extend the previous observations with adenine, to evaluate the effect of other purines (hypoxanthine and guanine), and of pyrimidine ribosides (cytidine and uridine), alone or in combination with adenine, on the preservation of erythrocyte viability throughout refrigerated storage.

Materials and Methods

Purine and Pyrimidine Compounds

Adenine sulfate (lot no. A89-70), hypoxanthine (lot no. H49-067) and guanine (lot no. G11-B-70) were obtained from the Sigma Chemical Co.; cytidine (lot no. 430845) and uridine (lot no. 105090) were obtained from the California Corporation for Biochemical Research. Solutions of adenine sulfate, cytidine and uridine were prepared in concentrations of 0.02 M in 0.15 M NaCl. Hypoxanthine and guanine were first dissolved in 1N NaOH, and then brought to 0.02 M with 0.15 M NaCl.

All solutions were sterilized through Morton ultrafine fritted Pyrex filters. The concentration of supplements was calculated by the absorption at 262 mμ (Amm 13.1, pH 1) for adenine sulfate, 250 mμ (Amm 10.6, pH 7) for hypoxanthine, 248 mμ (Amm 11.4, pH 1) for guanine, 280 mμ (Amm 13.0, pH 1) for cytidine, and 262 mμ (Amm 10.1, pH 7) for uridine.

The collection and storage of blood and the measurement of post-transfusion survival were performed as previously described. All supplements were added to the ACD*-blood at the beginning of the storage period. In some experiments blood was stored in glass containers; in the remaining studies plastic bags were used. The type of containers used...
in each experiment is indicated in the tables and figure. In each study donor variation was
eliminated by dividing a given donor unit into multiple test and control samples. Chromium\(\text{Cr}^{51}\) was employed for labeling stored cells; fresh cells, labeled with \(\text{P}^{32}\), served as a reference for the recipient's blood volume. Just before infusion approximately 1 ml.
of the \(\text{P}^{32}\)-labeled suspension of fresh cells was mixed with 10 ml. of the \(\text{Cr}^{51}\)-labeled
stored cell suspension. From this mixture approximately 5 ml. was injected into each of
two recipients, and the remaining cells were used for the preparation of radioactivity
standard solutions. Samples were obtained for radioactivity determinations 10 minutes,
1, 2, and 3 days after infusion of the labeled cells.

The theoretical 100 per cent survival, expressed as \(\text{Cr}^{51}\) counts, was determined from
the ratio of \(\text{Cr}^{51}\) and \(\text{P}^{32}\) radioactivity in the sample infused and from the \(\text{P}^{32}\) radio-
activity of the 10-minute post-transfusion specimen. The actual \(\text{Cr}^{51}\) activity in each post-
transfusion sample could then be expressed as per cent survival. Post-transfusion survival
at 0 time was calculated by averaging the survival values for days 1, 2, and 3, assuming
a rate of disappearance of radioactivity from the recipient's circulation of 2 per cent per
day after the initial loss of non-viable cells.\(^1,3\) Hemolysis occurring during storage amounted
to 1 per cent or less and was subtracted from the calculated survival figure. The value
thus obtained was taken as a measure of the actual post-transfusion survival of the stored
cells\(^3\) and is termed "viability." The viability figures listed are the average values obtained
in two recipients from each blood specimen. The standard deviation of the 78 duplicate
survival determinations included in this study was 2.2 per cent.\(^*\)

RESULTS AND DISCUSSION

In the following experiments the effect of purine or pyrimidine supple-
mentation was evaluated by measuring the post-transfusion viability of red
cells stored 6 weeks at 4 C.

Adenine—Optimal Concentration

Previous studies established that supplementation with 0.5 to 1.0 \(\mu\)M of
adenine (all supplements are presented as \(\mu\)M per ml. ACD-blood) would
maintain satisfactory viability for at least 5 weeks. To define more accurately
the optimal concentration, a comparison between a 0.5 and a 0.8 to 1.0 \(\mu\)M sup-
plement of adenine was undertaken (table 1). Nine donor units have been
studied (including two published previously\(^1\)). The difference in post-trans-
fusion viability between the lower and higher concentration of adenine
ranged from -3 per cent to +4 per cent, average +1 per cent. Thus, average
viability after 42 days of storage was 75 per cent with 0.8 to 1.0 \(\mu\)M of
adenine and 74 per cent with the 0.5 \(\mu\)M supplement. Viability of the unsup-
plemented controls averaged 49 per cent. It appears, therefore, that preserva-
tion with 0.5 \(\mu\)M of adenine per ml. of blood is as effective as with the larger
amounts tested.

Guanine

The occurrence of guanosine triphosphate (GTP) in human red cells has
been described\(^5-7\) but its function has not been elucidated. While adenine is
readily incorporated by human red cells into the adenine of ATP, it is a poor
precursor of the guanine of GTP.\(^7-11\) Guanine, on the other hand, is readily in-

\[\text{Standard deviation} = \sqrt{\frac{\sum d^2}{2n}} \]

where \(d\) is the difference between duplicates and
\(n\) the number of specimens.\(^4\)
ADENINE IN RED CELL PRESERVATION

Table 1.—Comparative Effect of Several Concentrations of Adenine and of Guanine and Cytidine with Adenine on the Viability of Red Cells Stored 42 Days in ACD

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Supplement</th>
<th>Post-Transfusion Viability (%)</th>
<th>Guanine (0.2)</th>
<th>Cytidine (0.3)</th>
<th>Adenine (0.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None 0.5</td>
<td>Adenine 0.8 1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B*</td>
<td>35 62</td>
<td>62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C*</td>
<td>39 64</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R*</td>
<td>50 68 72</td>
<td>81</td>
<td>83</td>
<td>86</td>
<td>88</td>
</tr>
<tr>
<td>S†</td>
<td>78</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T†</td>
<td>80 83</td>
<td>82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U†</td>
<td>84 86</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V†</td>
<td>62 80 82</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W†</td>
<td>53 78 75</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X†</td>
<td>52 75 75</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Survival results for the fresh controls were: Experiments B, 98 per cent; C, 98 per cent; R, 103 per cent; S, T, U, 100 per cent; and V, W, X, 98 per cent. Experiments B and C have been published previously.1

*Glass storage containers.
†Plastic storage containers.

The effect of guanine on red cell preservation was therefore determined. A single unit of blood was subdivided into multiple samples which were supplemented with guanine either alone or in combination with adenine (experiment R). Post-transfusion viability after 42 days of storage was then compared with the unsupplemented control (table 2). The lower amount of guanine (0.8 μM) appeared to exert a slightly beneficial effect on viability (58 per cent with guanine as compared with 50 per cent for the ACD control); this effect, however, was small when compared with 0.8 μM of adenine (viability 72 per cent). Guanine displayed no additive or synergistic effect with adenine in this experiment.

Hypoxanthine

Hypoxanthine accumulates in large amounts following the addition of inosine to the preservative medium.12-14 In view of the observation that large amounts of adenine are less effective than smaller supplements in preserving viability,1

Table 2.—Comparative Effect of Adenine and Guanine on the Viability of Red Cells Stored 42 Days in ACD

<table>
<thead>
<tr>
<th>Adenine Supplement</th>
<th>Guanine</th>
<th>Viability at 42 Days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM per ml. blood</td>
<td>Experiment R*</td>
<td>50 (51, 49)</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>58 (58, 57)</td>
</tr>
<tr>
<td>—</td>
<td>0.8</td>
<td>52 (53, 51)</td>
</tr>
<tr>
<td>0.8</td>
<td>—</td>
<td>72 (74, 70)</td>
</tr>
<tr>
<td>0.8</td>
<td>0.8</td>
<td>73 (73, 72)</td>
</tr>
</tbody>
</table>

Individual viability figures are listed in parentheses.
The survival result for the fresh control was 103 (103, 102) per cent.

*Glass storage containers.
it seemed possible that hypoxanthine in large amounts might also be toxic to the stored cells. If so, such an effect might partially obscure an otherwise beneficial action of inosine and thus account in part for the equivocal results which have been observed with inosine supplementation.\(^1,15\) Accordingly, the influence of varying amounts of hypoxanthine on post-transfusion viability was studied (experiment R, table 3). Viability, with or without hypoxanthine, averaged 50 per cent. Evidently, the presence of large amounts of hypoxanthine (10 \(\mu M\) per ml. blood) throughout the storage period has little influence on post-transfusion viability. It is doubtful, therefore, that hypoxanthine accumulation influenced the effectiveness of inosine as a preservative. On the other hand, the failure of hypoxanthine to affect favorably the maintenance of post-transfusion viability may relate to the observation that human erythrocytes are unable to convert hypoxanthine to adenine uridylate.\(^4,11\)

**Pyrimidine Riboside Supplementation**

Aronow\(^4\) has shown that while adenine may inhibit the growth of mammalian cells in tissue culture, this inhibitory effect could be overcome by adding small amounts of cytidine or uridine to the incubation medium. These results bear a superficial resemblance to our finding that larger amounts of adenine are less effective than smaller supplements in preserving viability. Accordingly, the effect of these compounds either alone or in combination with adenine on the preservation of red cell viability was examined. A single unit of blood was subdivided into multiple samples which were supplemented with adenine, cytidine, or uridine (experiment Q). Viability after 42 days of storage was compared with an unsupplemented sample (table 4). When adenine was present, viability averaged 83 per cent; without it, about 64 per cent of the cells survived. Neither cytidine or uridine in the concentrations used (0.8 \(\mu M\)), either alone or in combination with adenine, appeared to exert a significant effect, although the result with cytidine was somewhat equivocal.

Three additional experiments were performed in which a combination of cytidine, guanine, and adenine was compared with adenine supplementation alone (table 1). The amount of cytidine and guanine added was low (0.2 \(\mu M\) per ml. blood each). In these concentrations, guanine plus cytidine, in combination with adenine, did not enhance viability over that observed with adenine alone.

Our experience with adenine as the sole supplement, at an optimal concent-

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**Table 3.—The Effect of Hypoxanthine on the Viability of Cells Stored 42 Days in ACD**

<table>
<thead>
<tr>
<th>Hypoxanthine Supplement (\mu M) per ml. blood</th>
<th>Viability at 42 Days per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>----</td>
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</tr>
</tbody>
</table>

*Glass storage containers.

The survival result for the fresh control was 103 (103, 102) per cent.
Table 4.—The Effect of Cytidine and Uridine on the Viability of Red Cells Stored 42 Days in ACD

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Viability at 42 Days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td></td>
</tr>
<tr>
<td>Cytidine 0.8</td>
<td>81 (83, 79)</td>
</tr>
<tr>
<td>Uridine 0.8</td>
<td>88 (89, 86)</td>
</tr>
<tr>
<td>0.8 per ml. blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td></td>
</tr>
<tr>
<td>Cytidine 0.8</td>
<td>81 (82, 80)</td>
</tr>
<tr>
<td>Uridine 0.8</td>
<td>83 (84, 82)</td>
</tr>
<tr>
<td>0.8 per ml. blood</td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td></td>
</tr>
<tr>
<td>Cytidine 0.8</td>
<td>83 (84, 81)</td>
</tr>
<tr>
<td>Uridine 0.8</td>
<td></td>
</tr>
<tr>
<td>0.8 per ml. blood</td>
<td></td>
</tr>
</tbody>
</table>

The survival result of the fresh control was 102 (102, 101) per cent.

*Glass storage containers.

tration of 0.5 to 1 μM per ml. blood, now totals 30 samples from 14 donors (including six donor experiments published previously) studied in 60 recipients (fig. 1). After 39 to 46 days of storage (average 43 days), 75 per cent of cells preserved with adenine survived (range 62 to 86 per cent); by contrast, 47 per cent of the control cells stored in ACD remained viable (range, 29 to 64 per cent). Thus, supplementation with adenine enhanced post-transfusion viability by an average of 28 per cent after 6 weeks of storage; within a given donor unit the enhancement ranged from 17 to 37 per cent. These data suggest that adenine extends the period of useful storage to 5 or 6 weeks.

Comparison between Glass and Plastic Storage Containers

While the early experiments were carried out in glass storage containers, the more recent samples were stored in plastic. When the plastic units are compared with the ones stored in glass, it appears that better preservation is achieved with the lot of plastic containers used in this study (fig. 1). Thus, average viability after 42 days of storage with adenine was 80 per cent in plastic as compared with 71 per cent in glass. Comparable values for the ACD controls were 56 per cent in plastic and 44 per cent in glass. These studies cannot be considered definitive, since donor variability was not controlled in this comparison. They suggest, however, that more extensive comparisons between glass and plastic, carried out after 6 weeks of storage, are warranted.

After 6 weeks of storage, in vitro hemolysis amounted to slightly less than 1 per cent. No significant difference between the control and the adenine-supplemented samples was observed.

Summary

Supplementation of the ACD-preservative with small amounts of adenine (0.5 μM per ml. amounting to 37 mg. of the base or 56 mg. of adenine sulfate per 550 ml. unit of blood) preserved satisfactory viability (post-transfusion survival greater than 70 per cent) of stored human red cells for 5 to 6 weeks. In the concentrations used, the addition of guanine, cytidine or uridine, alone or in combination with adenine, had little or no effect in extending viability.
I 0-

Fig. 1.—Effect of duration of storage at 4 C. on the viability of red cells stored in ACD plus adenine. Fourteen donor units were studied. Six of these have been previously reported. At the outset of storage 0.75 ± 0.25 μM of adenine was added per ml ACD-blood. Other samples from the same units were not supplemented. Each point represents the average viability figure obtained in two recipients from each blood specimen (see Methods). The figure thus indicates the extent to which cells from different donors vary in their capacity to remain viable after refrigerated storage in comparable preservative media. The importance of eliminating the donor as a variable in the evaluation of a preservative—by dividing a given unit into test and control samples—is apparent.

Hypoxanthine, even in large amounts, did not appear to be toxic to the stored cells. Preservation of viability after 6 weeks of refrigerated storage may be somewhat improved by storage in certain plastic containers as compared with glass.

**SUMMARIO IN INTERLINGUA**

Le supplementation del preservativo ACD con micre quantitates de adenina (0,5 μM per ml, amontate a 37 mg del base o a 56 mg de sulfato de adenina per 550 ml de sanguine) resultava in un satisfacente preservation del viabilitate (superviventia post-transfusional de plus que 70 pro cento) de erythrocytos human magasinate durante 5 a 6 septimanas. In le concentrationes usate, le addition de guanina, cytidina, a uridina—sol o in combination con adenina—ha pauc o nulle effecto in le extension del viabilitate. Hypoxanthina, mesmo in grande quantitates, non pare esser toxic pro le magasinate cellulas. Le preservation del viabilitate post 6 septimanas de magasinage refrigerate es possiblemente meliorate un pauco si le sanguine es magasinate in recept-aculos de plastico in loco de vitro.
ACKNOWLEDGMENTS

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REFERENCES


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Red Cell Preservation: Further Studies with Adenine

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