

Eine Radiochrommarkierung von gelagerten Blutkonserven zur Bestimmung der Überlebenszeit ist nach dem vorgelegten Untersuchungsmaterial ohne weiteres möglich, da die Verhältnisse nicht von denen bei der Frischblutmarkierung abweichen. Allerdings sollte auf die Entfernung überschüssigen Radiochromats durch Waschen verzichtet und dieses durch Reduktion des sechswertigen Chroms zu dreiwertigem mittels Ascorbinsäure ersetzt werden. Bei der Beurteilung der Überlebenszeit retransfundierter gelagerter Erythrozyten sollte in jedem Falle vom 15-Minuten-Wert – oder einem vergleichbaren Wert – ausgegangen werden, da bei der Wahl des 24-Stunden-Werts als Ausgangsbasis der Lebenszeitkurve enorm überhöhte Werte ermittelt werden.

#### Summary

During studies on the possibility of storing blood, the survival time was determined for fresh and preserved blood. The method mainly used was the easy one of tagged radiochrome. Furthermore, detailed studies were also performed on the percentage of Cr<sup>51</sup> taken in and its bonds with erythrocytes, as well as on the possibility of using vitamin C (ascorbic acid) to interrupt assumption of Cr<sup>51</sup>. The survival rate was determined after transfusion of tagged erythrocytes, partly comparing with *Ashby's* technique. The results are reported.

#### Literaturverzeichnis

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2. *Gray, S. J.* and *Sterling, K.*: The tagging of red cells and plasma proteins with radioactive chromium. *J. clin. Invest.* 29: 1604 (1950).
3. *Read, R. C.*; *Wilson, G. W.* and *Gardner, F. H.*: The use of radioactive sodium chromate to evaluate the life span of the red blood cell in health and in certain hematological disorders. *Amer. J. med. Sci.* 228: 40 (1954).

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## Effect of Inosine on Red Cell Preservation

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The addition of inosine, a nucleoside purine, to acid-citrate-dextrose solution has been proposed as a means for prolonging the storage period of blood used for transfusion purposes. Inosine appears to affect the carbohydrate and phosphate metabolism of the erythrocyte and thereby improve the viability of stored red cells.

Since an increase in the outdating period of blood would be of great value, this study was undertaken by four different laboratories in order to compare acid-

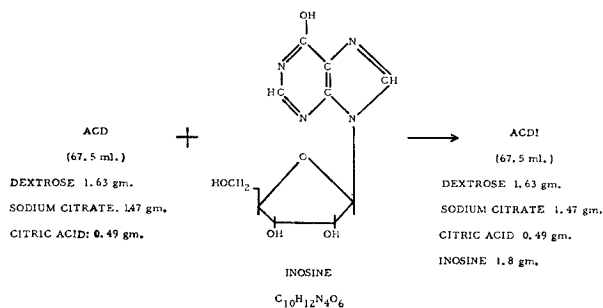


Fig. 1

citrate-dextrose (ACD) and acid-citrate-dextrose-inosine (ACDI) as blood preservatives. In 109 studies the comparison of ACD and ACDI was made by biochemical and hematologic tests done *in vitro* and the survival of transfused chromium 51-tagged red cells measured *in vivo*.

For each study approximately 450 ml of blood were collected from healthy human volunteers in either ACD or ACDI solution. If less blood was collected it is indicated in the figures. The first slide shows the chemical formula for inosine. Both preservative solutions contained 67.5 ml of ACD, NIH Formula A. For the ACDI solution, 1.8 g of inosine were added to the ACD solution before autoclaving. Both glass bottles and plastic packs were used as containers.

Following collection, the blood was stored at 4° C for periods of 20–43 days. At various time intervals, portions of the blood were tagged with radioactive chromium and transfused to the original donor or appropriate recipients, and the survival rate was determined. The blood volume of the recipient was determined by *Evans* blue dye, radioactive phosphorus or radioiodinated albumin.

In four studies the blood was stored for 21 or 28 days and after 2 weeks additional storage, the tests were repeated in the same subjects. In four studies the entire unit was transfused.

*In vitro* tests performed included determinations of mean corpuscular hemoglobin concentration, mean corpuscular volume, sodium, potassium, plasma hemoglobin, pH, lactic acid, dextrose, adenosine-triphosphate, and osmotic fragility.

Before transfusion, Gram stained smears were examined for bacterial contamination. None was found.

The *in vitro* results of sodium potassium and plasma hemoglobin are shown on the next slide. The days storage in each slide is given on the abscissa. The three types of containers used and the value of the determination are given on the ordinate. In each figure the value for storage in ACD is represented by an X or plus sign and for ACDI by a solid figure. Where 450 ml was transfused it is indicated by a circle around the dot and where two studies were performed the values are connected by a line.

Except for a slightly elevated plasma hemoglobin in units collected in glass bottles initially and on storage there was no significant differences between the

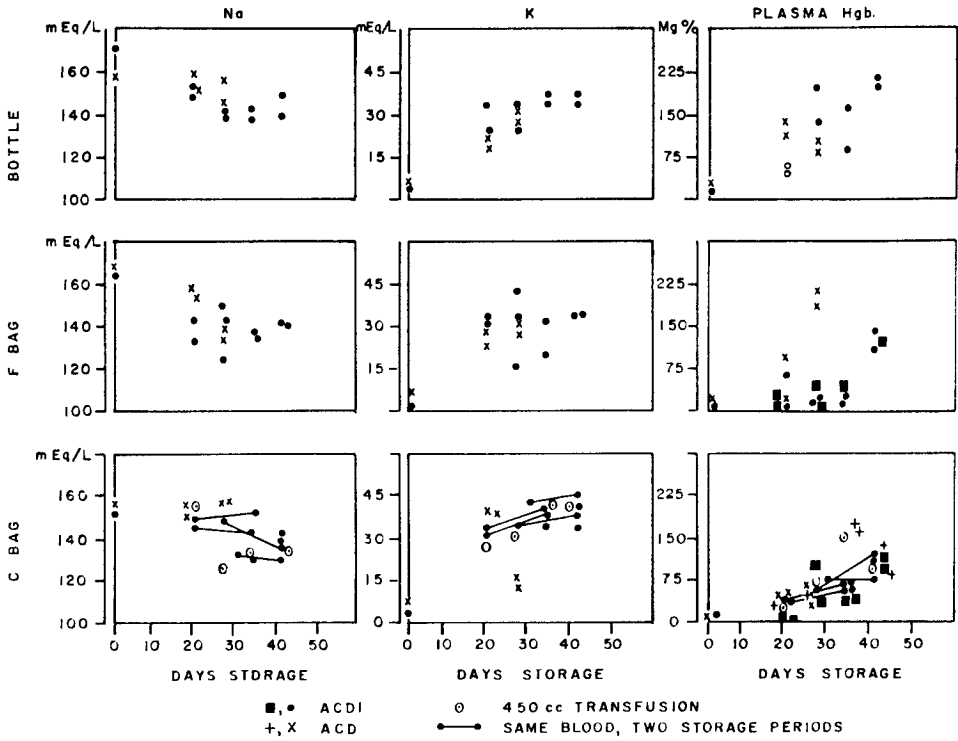


Fig. 2

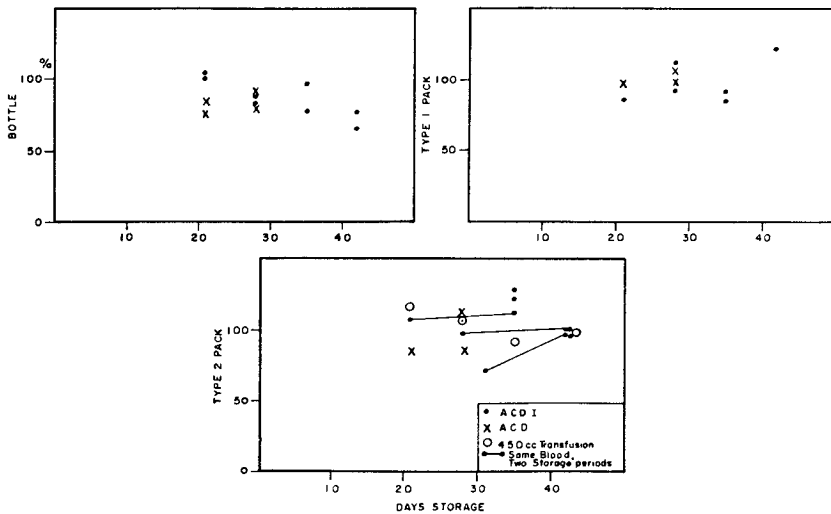


Fig. 3. 30' Survival (CA).

containers. A fall in plasma sodium concentration occurred during storage. Concurrently, there was an increase in plasma potassium. The plasma hemoglobin also increased. Variable results were obtained in the measurement of pH, lactic acid and dextrose. In 35 of 45 determinations the mean corpuscular hemoglobin concentration increased indicating the loss of some water. Inosine did not retard these *in vitro* changes. However, when adenosine triphosphate levels were measured, the blood preserved in inosine showed a higher amount of ATP than control bags not containing inosine.

As shown on the next slide *in vivo* there was little loss of red cells during the first 30 minutes after transfusion. There appeared to be a greater loss of cells stored in bottles but this was probably not significant.

The next slide graphically portrays the survival of cells at 24 hours. Only units stored in plastic packs are shown. The results when bottles were used as a container were similar. The percentage of survival at 24 hours is given on the ordinate. All of the units stored in ACD for 21 days showed at least 70 per cent survival at 24 hours. However, when storage was continued to from 35 to 43 days, the survival was quite variable. Six of 20 units – only three of the results are shown on this slide – stored

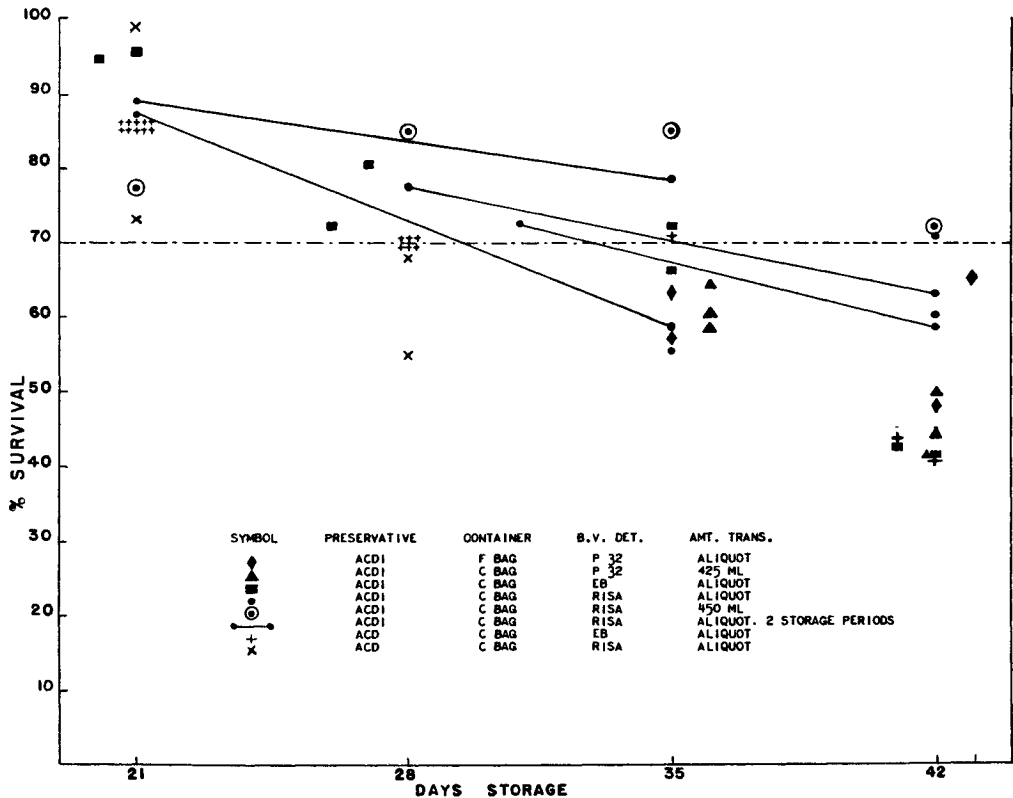


Fig. 4. Initial survival of red cells preserved in ACD1 + ACD.

35 days had a survival of better than 70 per cent. Only four of 23 units stored 42 or 43 days had a 70 per cent survival at 24 hours. Where duplicate studies were performed, the decreased 24-hour survival is readily apparent. The transfusion of 450 ml of blood as indicated by the circles did not effect the results *in vivo*.

In general, the viable cells survived well; however, as shown on the next slide the apparent half time survival decreased as the length of storage increased. The half time survival in days is given on the ordinate and the cross-hatched area indicates the normal value for unstored blood. Five of six stored in ACD had a T/2 of at least 25 days when stored for 28 days. Eight of nine units stored in ACDI for 28 days had a T/2 of greater than 25 days. However, all 14 units stored 42 days in ACDI had a T/2 of less than 25 days.

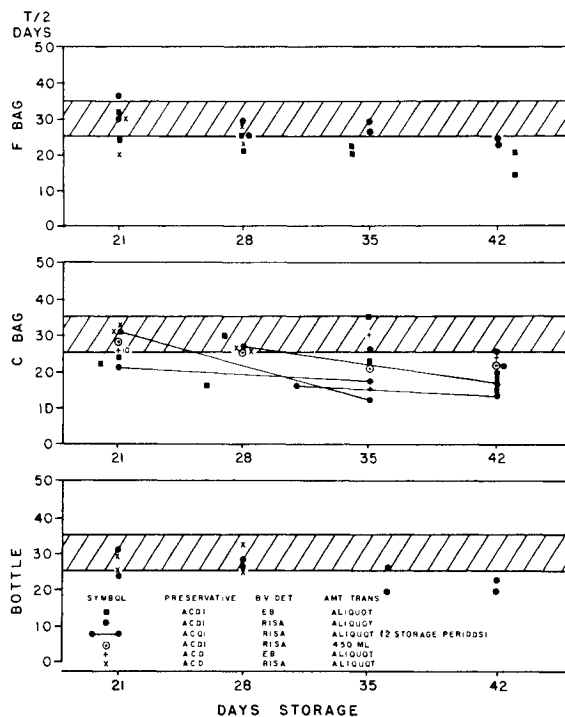


Fig. 5. T/2 of Cr<sup>51</sup> RBC survival of blood preserved in ACD +ACDI.

When the entire 450 ml of blood was transfused the pulse and blood pressure were not affected. In two instances the uric acid in a 24-hour collection of urine, following the transfusion, was found to be 1072 mg and 1940 mg. Normal values range up to 1000 mg.

These studies failed to confirm *Gabrio's* observation that with the addition of inosine that blood could be stored five or six weeks and still have an average survival of 82 per cent. There were certain technical differences in the two studies such as the method of sterilization, but from a chemical standpoint the inosine was assum-

ed to be the same. However, since some samples of blood stored in ACDI in the present study did give satisfactory survival results at 35 and 42 days, it has been suggested that red cells of different individuals may vary in their ability to withstand prolonged storage.

It should be noted that the purine nucleosides are metabolized to uric acid. While in healthy volunteers the transfusion of 450 ml of blood was without apparent toxicity, the effect of multiple transfusions is not known. The possible toxicity of these purine compounds is under investigation at this time.

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## Critères de conservation des globules rouges séparés : Application à la transfusion

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La transfusion des globules rouges séparés du plasma a pris depuis 10 ans un essor considérable dont témoignent de nombreuses publications\*. Les indications se sont de mieux en mieux précisées. L'accent est porté désormais moins sur l'économie substantielle ainsi réalisée que sur les qualités propres des hématies administrées avec ou sans lavage préalable.

Nous avons voulu vérifier si la limitation usuelle à 24 ou 48 h du délai de conservation des globules rouges séparés représente réellement un impératif.

Si ce délai pouvait être allongé, cela permettrait de constituer une « banque de culots globulaires » capables de satisfaire les demandes en fin de semaine ou pendant les jours fériés. Dans ce but nous avons étudié le devenir des globules rouges séparés du plasma et utilisés dans un délai de 3, 4 et 7 jours après le prélèvement.

Cette étude de la viabilité des culots globulaires en fonction du temps de conservation a été conduite selon 2 méthodes :

1<sup>o</sup> Etude chimique *in vitro* (recherche des critères de souffrance globulaire)

2<sup>o</sup> Etude de la survie *in vivo* des hématies marquées au Cr<sup>51</sup>.

Nous rappellerons que le sang est prélevé sur solution ACD et que nous tenons comme une règle formelle l'épreuve de compatibilité effectuée soit au Centre de Transfusion de l'Armée soit au service utilisateur.

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\* Une bibliographie importante consacrée à ce sujet se trouve dans un article récent de Maupin, Vigne et al. (Vox Sang. 1958).