Satisfactory Red Cell Viability with Slight Excess of Acid Citrate Dextrose

By Klaus Mayer and Joseph D'Amario

DONOR BLOOD for transfusion is collected in an acid-citrate dextrose (ACD) solution which has a pH of 5.0. When collected in the recommended portion of 100 ml. blood to 15 ml. ACD, the resultant pH is 6.8. Red cells from blood collected in this manner and refrigerated at 1–6°C are optimally preserved and may be transfused 21 days after collection.1

The increased demand for platelet concentrates focuses the need for a method of blood collection which results in optimal red cell and platelet viability.

It has been shown that platelet clumping is minimized and subsequent survival improved when the pH is lowered to 6.5,2,3,4,5,6 This acidification can be done in a number of ways, but the simplest approach is to collect proportionately less blood into a bag containing the standard amount (75 ml.) of ACD (N.I.H. formula A). Although the platelet product is improved in this way, the effect of the excess ACD on red cell viability after storage under blood bank conditions must be determined. An awareness that a large excess of ACD will impair red cell survival7 prompted us to test the survival of red cells stored in a much lesser excess of ACD for 21 days. These increases in the proportion of ACD have been limited to an amount which lowered the pH to 6.4 at 20°C. This study was not designed to test the effect of pH on platelets or other factors such as cryoprecipitate.

MATERIALS AND METHODS

The subjects for this study were volunteers from the staff of the Memorial-Sloan-Kettering Cancer Center. All subjects were hematologically normal and had not donated blood in the previous four months. They were instructed not to donate blood during the period of this study.

ACD (N.I.H. formula A) was removed from a plastic Blood Pack® through a sterile

From the Blood Bank, Department of Medicine, Memorial Hospital for Cancer and Allied Diseases, New York, N.Y.

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Klaus Mayer, M.D.: Director of Blood Bank, Memorial Hospital for Cancer and Allied Diseases; Director of Blood Bank, Hospital for Special Surgery, New York, N.Y.; Clinical Associate Professor of Medicine, Cornell University Medical College, New York, N.Y.

Joseph D'Amario, B.S.: Formerly with the Blood Bank, Memorial Hospital; now Medical Student, University of Leiden.

*Fenwal® JA-2C
Fenwal® AE-9
Rachromate, Abbot Laboratories

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### Table 1.—Results of $^{51}$Cr Survival Studies of Red Cells, Stored 21 Days. The Blood Was Collected in Various Amounts of ACD Which Corresponds to ACD/Blood Ratio in Parentheses

<table>
<thead>
<tr>
<th>SEX</th>
<th>ACD ml</th>
<th>BLOOD ml</th>
<th>STORAGE 4-6 C. days</th>
<th>BLOOD VOLUME ml/kg</th>
<th>pH 20 C.</th>
<th>15 min.</th>
<th>$^{51}$Cr Survival 1 hr.</th>
<th>2 hr.</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>T/2 days</th>
<th>T/10 days</th>
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<td>5 (75)</td>
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<td>21</td>
<td>71.9</td>
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medication injection site. It was transferred to a sterile, pyrogen-free siliconized 100 ml. bottle with a sterile disposable plastic syringe.

Twenty-five to thirty-two ml. of venous blood was collected without stasis using an 18 gauge needle and a sterile disposable 50 ml. syringe. The blood was transferred to a 100 ml. flask containing ACD. The volumes of blood and ACD were varied in order to obtain different pH. values. All blood-ACD mixtures were mixed by gentle rotation, centrifuged and the supernatant plasma was discarded. The cells were then stored at 4–6 C. for 21 days in a blood bank refrigerator.

After 21 days, the flasks were removed from the refrigerator and aliquots were taken for pH. determinations. From 200 to 250 µc. 51Cr as sodium chromate was added and allowed to stand at room temperature for 75 minutes. They were then centrifuged at 20 C. for 10 minutes at 2000 rpm. (1000 X G) in an International PR-2 centrifuge. The supernatant was removed and the cells were washed four times with sterile pyrogen-free 0.9 per cent sodium chloride. The hemoglobin content of supernatant plus washings was less than one per cent. After the last wash, the cells were reconstituted to a hematocrit of approximately 50 per cent. Following gentle mixing, aliquots were removed for use as standards and from 18 to 28 ml. was administered to the volunteer recipients. Each recipient received his or her own tagged cells.

Heparinized blood specimens were collected at 15, 60 and 120 minutes post-injection and then after 24 and 48 hours. Additional specimens were collected between the second and one hundredth day. The blood specimens were prepared and counted as previously described.7 Blood volumes were calculated from the counts obtained from the 15 minute specimens and from the standard counts. The values obtained fell within the expected limits of normal and thus the counts at 15 minutes post-injection were accepted as 100 per cent activity.

RESULTS

Table 1 depicts the results of 51Cr red cell survival data. The quantities of blood and ACD used are reduced but are proportional to the figures in parentheses. The blood volume calculated on the basis of radioactivity of injected erythrocytes was in all cases within the accepted normal range.1 The survival at 48 hours was 70 per cent or greater in each instance and there was no "collapse survival" as demonstrated by a retention of greater than 10 per cent of the red cell radioactivity for 76–97 days.

DISCUSSION

“The main criterion of successful red cell preservation is the normal survival of red cells after transfusion.”8 For a method of collection and storage to be acceptable it must be shown that at least 70 per cent of the transfused cells are viable.9 When 500 ml. blood is collected in 75 ml. ACD (N. I. H. formula A), this condition is readily met. This is the proportion of ACD to blood which is approved and recommended for routine blood bank practice.1 The pH. of the preserved blood is 6.7 at 22 C. When the proportion of ACD to blood is increased, the pH. is lowered as is desirable for collection of platelets. Therefore, to attain a pH. of 6.5 which is optimal for platelets it is only necessary to reduce the amount of blood drawn into a standard volume of ACD or to increase the ACD in the collection bag and add the standard amount of blood. This was done in the experiments listed. The fact that the calculated blood volume based on the 15 minute sample was normal has been interpreted to indicate that there was not an immediate destruction of the retransfused red
cells. Had there been a major loss of radioactivity within the first 15 minutes after injection the blood volume calculated would have been expanded proportional to the loss. This did not occur in any of the studies.

The data presented indicate that the slight excess ACD to blood does not adversely affect the red cell viability even after 21 day storage. The cells drawn in this proportion fare as well as those collected in the approved ratio.

**Summary**

Platelet suspensions obtained from blood donors are improved by increased acidification. The simplest way to accomplish this is to collect less blood into the standard quantity of ACD. Since we have previously reported an impairment in red cell viability when blood is collected in a great excess of ACD, it became pertinent to test the survival of stored red cells collected in a "slight" excess of ACD.

The volume of the blood collected was lowered to 375 ml. in 75 ml ACD (N. I. H. formula A). At this ratio the pH, was 6.5 which is sufficiently low to minimize platelet clumping. The red cells were separated, stored for 21 days at 4 C., and viability was tested by the 51Chromate method. The results showed adequate red cell survival for blood collected and stored in this manner.

**SUMMARIO IN INTERLINGUA**

Suspensiones plachettal obtenite ab donatores de sanguine es meliorate per un augmentate acidification. Le plus simple methodo pro obtener iste resultato es colliger minus sanguine ad in un quantitate standard de ACD. Viste que nos ha previemente reportate responsas adverse in le viabilitate erythrocytic quando le sanguine eseva colligate in un grande excesso de ACD, il eseva pertinentemente interprendere tests del superviventia de thesaurisate erythrocytos colligate in un "leve" excesso de ACD.

Le volumine del sanguine colligate eseva reducite a 375 ml in 75 ml ACD (formula A del N.I.H.). Sub iste conditiones, le pH eseva 6,5 lo que es sufficientemente basso pro reducir al minimo le congregage del plachettas. Le erythrocytos eseva separate, thesaurisate durante 21 dies a 4 C, e le viabilitate eseva testate per le metodo a chromato a 51Cr. Le resultatos monstrava adequate superviventias pro le sanguine colligate e thesaurisate in iste maniera.

**REFERENCES**

1. Biological Products, Public Health Service Regulations, Title 42, Part 73.302.
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