

Red Cells Preserved with 10% Hydroxyethyl Starch: Effect of Prefreeze Washing

L. WEATHERBEE, E. D. ALLEN, H. H. SPENCER, S. M. LINDENAUER,
AND P. A. PERMOAD

*Veterans Administration Hospital and University of Michigan Medical Center,
Ann Arbor, Michigan 48105*

Hydroxyethyl starch (HES) has shown promise as an extracellular cryoprotective agent for red blood cells. Since it is non-toxic and red cells are osmotically stable in its presence, a thawed unit could be administered without removing the protective agent. The most effective concentration of HES for red cell cryopreservation is 14% (2-4). Concentrations higher than this are extremely viscous when mixed with red cells making handling difficult. Lower concentrations generally give poorer preservation with respect to the postthaw parameters usually examined (saline stability, red cell recovery, and supernatant hemoglobin levels).

An exception to this finding is that 10% HES consistently yields higher saline stabilities (indicating less damage) than 14% HES, although the values obtained for cell recovery and levels of supernatant hemoglobin suggest greater damage. Thus, despite the advantages of the 10% HES concentration (less starch, lower viscosity, faster transfusion) the higher levels of supernatant hemoglobin have made it unacceptable.

Recently, we reported that red cells washed with saline before freezing with 14% HES yielded increased saline stabilities while retaining low levels of supernatant hemoglobin and high cell recoveries (4). This paper describes the effect of

several prefreeze wash solutions to determine whether a similar procedure could improve the quality of 10% HES-preserved red cells.

MATERIALS AND METHODS

Blood (CPD collected, 2-15 days old) was used for all experiments. A single lot of powder starch (McGaw No. 9330) obtained from McGaw Laboratories, Glendale, CA was used as a 40% solution. Aliquots of 30 ml of the red cell-HES mixture were frozen (liquid nitrogen) and thawed (water, 48°C) in Hemoflex bags as previously described (2, 4).

Washed cells are those prepared in the following manner. Whole blood is centrifuged (3000 rpm, 15 min) and the supernatant plasma and buffy coat are removed. Wash solution is added to a volume equal to that of the centrifuged cells, the cells are mixed and centrifuged as before. After removing the supernatant, the procedure is repeated a second time.

Unwashed cells are those from which supernatant plasma and buffy coat are removed from whole blood after centrifugation (3000 rpm, 15 min).

Postthaw evaluation of the red cells included determination of saline stability, supernatant hemoglobin levels, and cell recovery as described earlier (4).

Received November 21, 1974.

TABLE 1
POSTTHAW STUDIES OF 10% HYDROXYETHYL STARCH-PRESERVED RED CELLS

| Wash solution before freezing with 10% HES | Hematocrit | Cell recovery (%) | Saline stability (%) | Supernatant hemoglobin (mg/100 ml) | No. units frozen |
|--|------------|-------------------|----------------------|------------------------------------|------------------|
| None (14% HES control) | 40 | 97.4 ^a | 75.1 ^a | 317.5 ^a | 16 |
| 0.9% NaCl (14% HES control) | 40 | 97.5 | 82.9 | 306.5 | 13 |
| None (10% HES control) | 39.5 | 97.1 | 80.3 | 461 | 17 |
| 0.9% NaCl | 40.5 | 95.9 | 76.3 | 829.4 | 15 |
| 0.7% NaCl | 41 | 94.4 | 77.5 | 1120 | 3 |
| 3% HES | 40 | 96.5 | 76.9 | 699 | 4 |
| 5% HES | 39 | 96.8 | 79.0 | 581 | 4 |
| Normosol | 40 | 96.0 | 74.5 | 753 | 2 |
| Normosol + 1% Dextrose | 41 | 96.0 | 76.8 | 784 | 3 |

^a Values given in the table represent the mean for the number of units frozen.

RESULTS

Postthaw parameters obtained when freezing red cells with 10% HES are shown in Table I. Results obtained with 14% HES on washed and unwashed red cells are also included to permit comparison. The lower cell recoveries and higher levels of supernatant hemoglobin obtained with unwashed cells frozen with 10%

HES are less satisfactory than values obtained with 14% HES preserved red cells. However, the saline stability of the 10% HES preserved red cells (unwashed) is higher than that of 14% HES preserved unwashed red cells.

When the cells are washed with saline (0.9% NaCl) before freezing with 10% HES the saline stabilities do not increase.

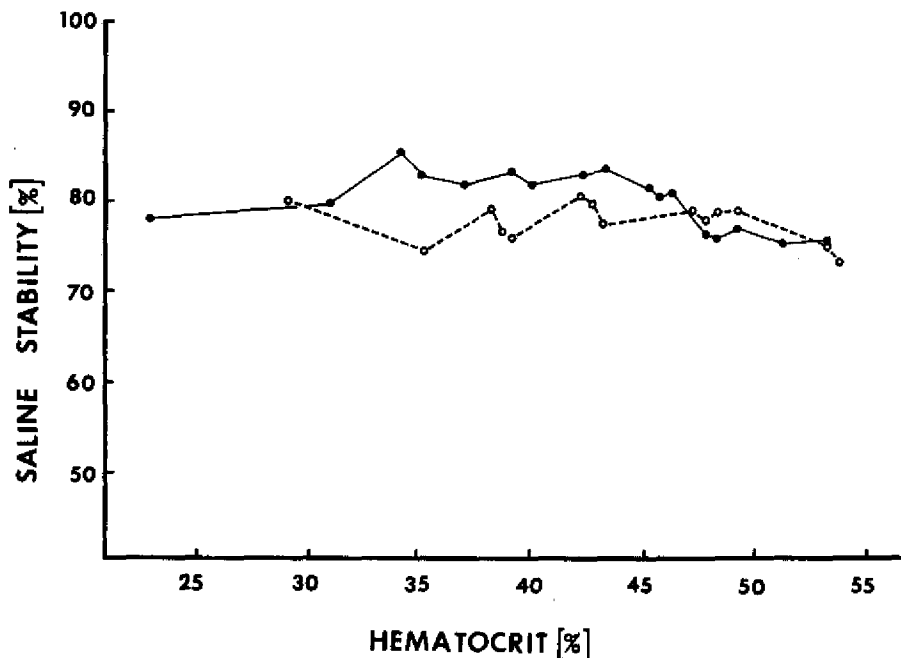


FIG. 1. Relationship of saline stability to hematocrit of 10% HES-preserved red cells. Each point on the curve represents the mean for several units frozen at that hematocrit. Prefreeze washed (—○—). Prefreeze unwashed (—●—).

Similarly, the cell recoveries are lower and the levels of supernatant hemoglobin are almost twice as high as those of the unwashed cells.

Other prefreeze wash solutions examined to determine whether any were capable of improving cell preservation with 10% HES are also shown in the table. These wash solutions appeared to uniformly reduce cell recoveries slightly and increase levels of supernatant hemoglobin. As shown in the table, a lower concentration of sodium chloride (0.7%) reduces cell recoveries to 94% and increases the levels of supernatant hemoglobin to over 1000 mg/100 ml. None of these other prefreeze wash solutions gave indications that the saline stability, the red cell recovery, or the level of supernatant hemoglobin would approach values obtained with 14% HES. Therefore, they were not examined beyond the preliminary observations shown in the table.

The units examined in Table 1 were frozen at hematocrits near 40. Since it was shown with 14% HES that the saline stability varied with the hematocrit at which the red cells were frozen (4), this was also examined with 10% HES. Unwashed red cells have saline stabilities above 80% throughout most of the hematocrit range (Fig. 1). Only at the lower (<25%) or the higher (>45%) hematocrit ranges do the saline stabilities drop below 80%. Washing does not improve the saline stability but instead reduces it to levels below unwashed cells throughout most of the hematocrit range. Only at the highest hematocrits examined (>47%) do the saline stabilities appear the same or slightly better than those of unwashed cells. However, at those hematocrits, the cell recoveries are lower and the supernatant hemoglobin levels higher than those of the unwashed cells (data not shown).

DISCUSSION

Cryopreservation with 10% HES has been paradoxical in that the saline stabi-

ties have been higher than with 14% HES and yet the cell recoveries and levels of supernatant hemoglobin have indicated poorer preservation. Ten percent HES would be more acceptable because of the reduced viscosity, faster transfusion, and decreased amount of cryopreservative required.

Prefreeze washing with saline improves the saline stability of 14% HES-preserved red cells but does not do so with 10% HES preserved cells. Preliminary examination of other wash solutions showed a similar trend with 10% HES. In fact, the data suggest that all three postthaw parameters indicate greater damage after prefreeze washing of 10% HES-preserved red cells.

The reason that the saline stabilities of unwashed cells preserved with 10% HES are often higher when compared to those frozen with 14% HES is not known. After prefreeze washing, however, the saline stabilities of cells frozen with 10% HES are reduced and thus the values of all three postthaw parameters represent greater damage than that obtained with washed red cells and 14% HES. These findings, considered as a whole, give further support to the selection of 14% as the optimum concentration of HES for cryopreservation of red blood cells.

SUMMARY

Prefreeze washing of red cells with saline and other wash solutions before freezing with 10% hydroxyethyl starch does not yield an improved product after thawing. The saline stabilities and cell recoveries are reduced and the level of supernatant hemoglobin increased. These data further support the use of 14% as the most acceptable concentration of hydroxyethyl starch for cryopreservation of red cells.

ACKNOWLEDGMENT

This work was supported in part by the Office of Naval Research, Department of the Navy. (Contract No. NAonr-8-75).

REFERENCES

1. Knorpp, C. T., Merchant, W. R., Gikas, P. W., Spencer, H. H., and Thompson, N. W. Hydroxyethyl starch; extracellular cryoprotective agent for erythrocytes. *Science* **157**, 1312-1313 (1967).
2. Knorpp, C. T., Starkweather, W. H., Spencer, H. H., and Weatherbee, L. The preservation of erythrocytes at liquid nitrogen temperatures with hydroxyethyl starch: the removal of hydroxyethyl starch from erythrocytes after thawing. *Cryobiology* **8**, 511-516 (1971).
3. Robson, D. C. Rapid freezing and thawing techniques using extra-cellular agents. In "Modern Problems of Blood Preservation" (W. Spielman, and S. Seidl, Eds.), pp. 199-203. Fischer Verlag, Stuttgart, 1970.
4. Weatherbee, L., Allen, E. D., Spencer, H. H., Lindenauer, S. M., and Permoad, P. A. The effect of plasma on hydroxyethyl starch-preserved red cells. *Cryobiology* **12**, 119-122 (1975).