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Rates of Hemolysis in Human Blood Stored in Dextrose Solutions and in Other Mixtures.

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One of the chief criteria for determining the suitability of preserved blood for transfusion is the extent of spontaneous hemolysis during storage. The maximal safe period of storage, therefore, depends upon the rate of hemolysis. Long storage is especially desirable in small blood banks and in the adaptation of stored blood for military and civilian emergencies.[1] It has been shown[2, 3] that hemolysis in human blood stored at 2°C begins almost immediately and proceeds at varying rates depending upon the preservative used. This paper presents data to show that the Rous-Turner dextrose-citrate mixture as modified by DeGowin, Harris, and Plass[2] is particularly adapted to long storage of human blood.

In these experiments, blood was drawn into appropriate volumes of 3.2% sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) in water, the exact dilution being estimated by the hematocrit method of Van Allen. Under aseptic technic, other solutions were added in accurate proportions and the resultant mixtures apportioned into cotton-stoppered flasks and stored at 2°C. At suitable intervals, flasks from each series were removed from storage and the plasmas separated by centrifugation. The plasma hemoglobin was determined by the Wu method[4] and the percentage of hemolyzed erythrocytes was calculated from the plasma hemoglobin value and the hematocrit reading.

Table I contains the data from representative experiments. During the first 5 days of storage hemolysis was minimal in all mixtures, but beyond that point there was considerable variation. Dilution with electrolytes (NaCl, KCl) provoked the most rapid hemolysis, and blood-citrate hemolyzed nearly as fast. Dilution with isotonic dextrose and sucrose solutions greatly inhibited hemolysis, dextrose being more efficient than sucrose. The blood-dextrose-citrate mix-

1 DeGowin, E. L., and Hardin, R. C., *War Medicine*, 1941, **1**, 326.

2 DeGowin, E. L., Harris, J. E., and Plass, E. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 126.

3 DeGowin, E. L., Harris, J. E., and Plass, E. D., *J. Am. Med. Assn.*, 1940, **114**, 850.

4 Wu, H., *J. Biochem.*, 1922, **2**, 189.

TABLE I.
Rates of Hemolysis in Human Blood Stored at 2°C in Various Preservative Mixtures. Values Expressed as Percent of Erythrocytes Hemolyzed.

Experiment No.	20A	18A	20B*	22A*	18C	20D	21A	20C	21B
Blood Mixture (vol.)									
Blood	23	23	10	10	10	10	10	10	10
Sodium Citrate 3.2%	2	2	2	2	2	2	2	2	2
Dextrose 5.4%	—	—	13	13	—	—	—	13	—
Sucrose 10%	—	—	—	—	13	—	—	—	—
Sucrose 4.5%	—	—	—	—	—	13	—	—	—
NaCl 0.9%	—	—	—	—	—	—	13	—	—
NaCl 0.5%	—	—	—	—	—	—	—	sol	—
KCl 1.15%	—	—	—	—	—	—	—	—	13
Percent Hemolysis									
Days storage									
0	0	0	0	0	0	0	0	0	0
5	0.09	0.17	—	.03	.03	0.03	—	.05	—
10	—	0.73	—	.08	.10	—	—	—	—
15	1.51	—	.08	.09	.22	1.14	2.64	.29	1.92
20	3.58	3.24	—	—	.44	1.01	—	—	—
25	7.20	—	.38	.21	.60	—	21.80	.62	19.10
30	13.80	9.08	—	.27	.85	2.57	—	—	—
35	—	—	.47	.43	—	—	—	.91	—
40	—	—	—	.92	—	6.75	—	—	—
45	—	—	.63	—	—	—	—	2.05	—

*Modified Rous-Turner mixture (DeGowin, Harris, and Plass).
sol—Dextrose dissolved in NaCl solution instead of distilled water. Otherwise sugars were dissolved in distilled water.

ture of DeGowin, Harris and Plass resulted in only 2-4% as much hemolysis in 30 days as did blood-citrate. In blood-dextrose-citrate there was less than ½ as much hemolysis in 30 days as occurred in blood-citrate in 10 days. These observations confirm the qualitative studies of Rous and Turner.[5]

The superiority of preservative mixtures containing dextrose has now been widely recognized. Many modifications of the Rous-Turner mixture[6-9] have been devised to reduce the final volume of the blood mixture by decreasing the volume of isotonic dextrose solution or by using hypertonic concentrations of the sugar. In each case the resulting dextrose concentration has been less than the approximate 3% recommended by Rous and Turner and by DeGowin, Harris, and Plass.

Table II presents data upon the effect of varying concentrations of dextrose (1.0-6.0%) on the rate of hemolysis during storage, when

[5] Rous, Peyton, and Turner, J. R., *J. Exp. Med.*, 1916, **23**, 219.

[6] Gwynn, C. A., and Alsever, J. B., *Am. J. Med. Sc.*, 1939, **198**, 634; Alsever, J. B., and Ainslie, R. B., *N. Y. State J. Med.*, 1941, **41**, 126.

[7] Maizels, M., and Whittaker, N., *Lancet*, 1940, **1**, 590.

[8] Aylward, F. X., Mainwaring, B. R. S., and Wilkinson, J. F., *Lancet*, 1940, **1**, 685.

[9] Barton, F. E., *N. England J. Med.*, 1941, **225**, 176.

the sodium citrate content was kept constant. Reduction of the final concentration of dextrose in the blood mixture below 3% resulted in increasing the hemolysis in 30 days of storage. Utilization of dextrose solutions of stronger than 10% did likewise. Other difficulties encountered in the use of hypertonic dextrose solutions are discussed elsewhere.

Summary. The addition of dextrose to blood-citrate mixtures markedly inhibits hemolysis during storage at 2°C. Sucrose is not so effective and NaCl and KCl increase the rate of hemolysis. The final concentration of dextrose in the blood mixture required for the maximal inhibition of hemolysis is approximately 3%. The use of a preservative mixture containing this amount of dextrose ordinarily permits the safe storage of blood for 30 days or more.

TABLE II.

Hemolysis in Blood-Dextrose-Citrate Mixtures After Storage for 30 Days at 2°C. Concentration of Dextrose Was Adjusted by Varying Volume and Concentration of Sugar Solutions. Concentration of Sodium Citrate Remained Constant at 0.26% (with exception of 19A1 and 19B1).

Experiment	Human blood (vol.)‡	Dextrose sol. added (vol.)	Dextrose sol. added (conc., %)	3.2% sodium citrate (vol.)	Dextrose conc. in mixture, %	Erythrocytes hemolyzed in 30 days (2 exper.) %
19B1	40	66.4	10.0	26.4	5.7	.29, .40
19B2	40	52.0	10.0	8.0	6.0	.25, .32
19B3	40	40.0	10.0	6.8	5.5	.25, .43
19B4	40	30.0	10.0	6.0	4.7	.28, .24
19B5	40	20.0	10.0	4.8	3.6	.33, .41
19B6	40	10.0	10.0	4.0	2.1	.64, 1.42
19A1*	40	66.4	5.4	26.4	3.0	.22, .27
19A2†	40	52.0	5.4	8.0	3.2	.20, .43
19A3	40	40.0	5.4	6.8	2.9	.31, .92
19A4	40	30.0	5.4	6.0	2.4	.47, .50
19A5	40	20.0	5.4	4.8	1.8	.97, 1.18
19A6	40	10.0	5.4	4.0	1.3	1.78, 2.54
23A1	35.7	10.7	23.3	3.5	5.0	.64, .58
23A2	35.7	10.7	18.6	3.5	4.0	.58, .41
23A3	35.7	10.7	14.0	3.5	3.0	.83, .49
23A4	35.7	10.7	9.3	3.5	2.0	1.87, .93
23A5	35.7	10.7	4.6	3.5	1.0	6.69, 2.70
23B1†	43	56.0	5.5	7.8	2.9	.26, .21
23B2	43	44.0	6.3	6.6	3.0	.28, .25
23B3	43	36.0	6.9	6.0	2.9	.32, .25
23B4	43	27.0	8.0	5.4	3.0	.40, .26
23B5	43	20.0	9.9	4.8	3.0	.45, .24
23B6	43	13.0	13.9	4.2	3.0	.82

*Rous-Turner mixture.

†Modified Rous-Turner mixture (DeGowin, Harris, and Plass).

‡Under the conditions of collecting blood the volume of the blood could only be approximated. This accounts for the disparity between calculated final concentrations of dextrose and actual values which are given in the 6th column.