

### Osmotic Changes in Erythrocytes of Human Blood During Storage.

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Scant attention has been given to osmotic changes occurring in erythrocytes stored at low temperatures although most workers insist upon the use of isotonic solutions in the preservative fluid. Rous and Turner<sup>1</sup> found no relationship between the resistance of red cells to hypotonic saline solutions and to mechanical shaking. Several investigators<sup>2-5</sup> have reported increased fragility of the erythrocytes during storage. Maizels and Whittaker<sup>6</sup> noted that although blood stored in hypertonic saline solutions showed little hemolysis the erythrocytes were ruptured when placed in normal plasma because their contents had become equilibrated to the hypertonic plasma. The permeability of red cells to dextrose suggested the possibility of a similar reaction when that sugar is used in the preservative. The present study is concerned with certain phases of this problem.

Table I presents the data from 3 representative experiments. Changes in cell volume were calculated from hematocrit readings. The fragility of the erythrocytes was estimated by adding 0.1 cc of whole blood mixture to 2.0 cc of each of a series of sodium chloride solutions with concentrations ranging from 0.24% to 1.20% in increments of 0.04%. After 1 hour at room temperature, the highest concentrations showing initial and complete hemolysis were noted—values are expressed in that order. For testing washed cells the plasma was removed after centrifugation and the cells washed twice with 0.95% sodium chloride solution before being added to the series of saline solutions.

In citrate-blood there was a progressive increase in cell volume during storage which was approximately paralleled by increase in fragility of both washed and unwashed cells. In dextrose-citrate-

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<sup>1</sup> Rous, Peyton, and Turner, J. R., *J. Exp. Med.*, 1916, **23**, 219.

<sup>2</sup> Doepp, M., *Deutsch. Z. f. Chir.*, 1934, **243**, 736.

<sup>3</sup> Belk, W. P., Henry, N. W., and Rosenstein, F., *Am. J. Med. Sc.*, 1939, **198**, 631.

<sup>4</sup> Kolmer, J. A., and Howard, Mary, *Am. J. Med. Sc.*, 1940, **200**, 311.

<sup>5</sup> Muehler, R. O., and Andrews, K. R., *Am. J. Clin. Pathol.*, 1941, **11**, 314.

<sup>6</sup> Maizels, M., and Whittaker, N., *Lancet*, 1940, **1**, 590.

TABLE I.  
Changes in Cell Volume and Fragility of Human Blood Stored at 2°C in Various Preservative Mixtures.

|                                       | Days of storage at 2°C |         |         |         |         |         |         |
|---------------------------------------|------------------------|---------|---------|---------|---------|---------|---------|
|                                       | 0                      | 5       | 10      | 15      | 20      | 25      | 30      |
| <b>Blood + Citrate 20A</b>            |                        |         |         |         |         |         |         |
| % change in cell volume               | 0                      | 0       |         | +13.8   | +21.4   | +31.5   | +47.0   |
| Fragility Unwashed                    | .40-.32                | .64-.32 |         | .64-.32 | .88-.40 | .88-.40 | .92-.44 |
| Washed                                | .40-.32                | .64-.36 |         | .64-.36 | .88-.40 | .88-.40 | .92-.44 |
| <b>Blood + Dextrose + Citrate 22A</b> |                        |         |         |         |         |         |         |
| % change in cell vol.                 | +53.0                  | +34.1   | +38.2   | +41.0   | +43.0   |         | +50.0   |
| Fragility Unwashed                    | .80-.64                | .76-.56 | .76-.56 | .80-.60 | .84-.60 | .80-.64 | .84-.64 |
| Washed                                | .44-.32                | .36-.24 | .36-.24 | .36-.24 | .36-.24 | .40-.28 | .36-.28 |
| <b>Blood + Sucrose + Citrate 20D</b>  |                        |         |         |         |         |         |         |
| % change in cell vol.                 | +21.4                  | -4.5    |         | -5.9    | -9.2    |         | -5.2    |
| Fragility Unwashed                    | .40-.28                | .36-.24 |         | .44-.24 | .52-.24 |         | .56-.24 |
| Washed                                | .40-.32                | .40-.24 |         | .48-.24 | .52-.24 |         | .64-.24 |

20A = 23 volumes blood + 2 volumes 3.2% sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) in water.

22A = 10 volumes blood + 2 volumes 3.2% sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) in water  
+ 13 volumes 5.4% dextrose in water.

20D = 10 volumes blood + 2 volumes 3.2% sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) in water  
+ 13 volumes 4.5% sucrose in water.

blood<sup>7</sup> the changes were more complicated. Within 1 hour after the addition of dextrose the cell volume had increased over 50%. During the first 5 days of storage the swelling diminished slightly but again gradually approached a maximum in 30 days. These changes were reflected in the high fragility for unwashed cells. Washed cells, however, showed normal fragility indicating that the swelling was due to the diffusion of dextrose and water into the erythrocytes and that this process was reversible.

Sucrose was used in hypotonic concentration since previous observation had shown that the cells shrank in 10% solution. In the recorded experiment with sucrose there was an initial increase in cell volume followed within 5 days by persistent shrinkage. The fragility of both washed and unwashed cells was well correlated with volume changes. The rapid change during the first 5 days was associated with marked loss of cellular potassium.

There was no direct correlation between the amount of cellular swelling and the extent of hemolysis during storage.<sup>7</sup> Dextrose-citrate-blood showed the greatest swelling and the least hemolysis while sucrose-citrate-blood underwent moderate hemolysis with shrunken cells. Citrate-blood, showing slightly less swelling than dextrose-citrate-blood, was greatly hemolyzed after 30 days.

<sup>7</sup> DeGowin, E. L., Harris, J. E., and Bell, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1942, **49**, 481.

It has been shown previously<sup>7</sup> that a final concentration of approximately 3% dextrose is optimal for the inhibition of hemolysis during storage but the total volume of the modified Rous-Turner mixture has militated against its wide acceptance. Table II presents the changes in cell volume and in fragility of blood stored in various concentrations of dextrose. Hypertonic dextrose concentrations induced less swelling in the erythrocytes during storage than did more nearly isotonic solutions. The cell contents became equilibrated to the hypertonic plasma so that the cells ruptured when placed in isotonic saline. Washing the cells in isotonic saline, however, resulted in normal fragility values for the cells which did not rupture, indicating that the diffusion of dextrose and water was responsible for the hypertonicity originally observed.

The gross appearance of blood stored in hypertonic dextrose is excellent but it is dangerous to ignore the hypertonicity of the erythrocytes. Serious intravascular hemolysis may result when such blood is transfused. Of 128 stored bloods (13 volumes of 10% dextrose, 2 volumes of 3.2% sodium citrate, and 10 volumes of

TABLE II.

Changes in Cell Volume and Fragility of Human Blood Stored at 2°C for 30 Days in Various Concentrations of Dextrose. The Concentration of Sodium Citrate Was Uniformly 0.26%.

| Experiment | Blood (vol.) | Dextrose sol. added |            | Dextrose conc. in mixture, % | Values after 30 days (2 experiments) |                             |
|------------|--------------|---------------------|------------|------------------------------|--------------------------------------|-----------------------------|
|            |              | (vol.)              | (conc., %) |                              | Change in cell vol., %               | Fragility of unwashed cells |
| 23A1       | 35.7         | 10.7                | 23.3       | 5                            | +14.6                                | 1.20-.72                    |
|            |              |                     |            |                              | +11.1                                | 1.20-.68                    |
| 23A2       | 35.7         | 10.7                | 18.6       | 4                            | +17.6                                | 1.10-.72                    |
|            |              |                     |            |                              | +20.9                                | 1.20-.72                    |
| 23A3       | 35.7         | 10.7                | 14.0       | 3                            | +23.6                                | 1.10-.68                    |
|            |              |                     |            |                              | +29.1                                | 1.10-.64                    |
| 23A4       | 35.7         | 10.7                | 9.3        | 2                            | +30.0                                | .96-.68                     |
|            |              |                     |            |                              | +40.5                                | 1.00-.56                    |
| 23A5       | 35.7         | 10.7                | 4.6        | 1                            | +32.0                                | .96-                        |
|            |              |                     |            |                              | +44.3                                | .88-.48                     |
| 23B1*      | 43           | 56                  | 5.5        | 2.9                          | +40.6                                | .80-                        |
|            |              |                     |            |                              | +28.6                                | .80-.52                     |
| 23B2       | 43           | 44                  | 6.3        | 3                            | +26.9                                | .84-.68                     |
|            |              |                     |            |                              | +28.8                                | .80-.52                     |
| 23B3       | 43           | 36                  | 6.9        | 2.9                          | +28.2                                | .88-.68                     |
|            |              |                     |            |                              | +24.8                                | .84-.52                     |
| 23B4       | 43           | 27                  | 8.0        | 3                            | +23.1                                | .88-.68                     |
|            |              |                     |            |                              | +25.3                                | .88-.52                     |
| 23B5       | 43           | 20                  | 9.9        | 3                            | +19.4                                | .96-.68                     |
|            |              |                     |            |                              | + 0.3                                | 1.00-.60                    |
| 23B6       | 43           | 13                  | 13.9       | 3                            | +33.8                                | 1.10-.68                    |

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

blood), 16 produced post-transfusion hemoglobinuria (one specimen had been stored only 3 days), 27 were discarded because the cells hemolyzed in 0.9% saline, and 85 were transfused without detectable hemoglobinuria. Whenever unwashed cells were hemolyzed by 0.9% saline they were also ruptured in fresh human plasma. Incompatibility from isohemagglutinins and isohemolysins was excluded in the cases studied. Evidently the osmotic activity of stored red cells must be considered in determining the suitability of preserved blood for transfusion.

It has been observed<sup>8</sup> that hemolysis frequently occurs after a few hours' refrigeration when large quantities of blood (500 cc) are introduced into warm (room temperature) dextrose-citrate mixture (modified Rous-Turner) but that effect is avoided if the mixture is ice-cold. Early hemolysis, moreover, does not occur when small quantities of the blood mixture are employed, a fact which suggests that the rate of cooling is important.

To test this hypothesis, blood was drawn into flasks of dextrose-citrate (modified Rous-Turner) at room temperature (27°C). After 2, 60, 120, and 210 minutes the erythrocyte fragility was determined and specimens were quickly chilled to 0°C. Initial hemolysis occurred at 0.44%, 0.76%, 0.84%, and 0.88% sodium chloride solution, respectively. Specimens which were chilled after 2 hours or more at room temperature showed considerable hemolysis, whereas the controls kept at room temperature did not hemolyze. Apparently, dextrose and water diffused into the cells at room temperature and the reduction in temperature ruptured the swollen erythrocytes. Jacobs, Glassman, and Parpart<sup>9</sup> report that as the temperature of an erythrocyte suspension is reduced, initial hemolysis occurs in progressively higher concentrations of hypotonic saline solution. The mechanism by which the quick chilling arrests the diffusion of dextrose and water into the cells is obscure but the empirical fact is important in the collection of dextrose-citrate-blood (modified Rous-Turner) for transfusion.

*Summary.* In citrate-blood the erythrocytes undergo progressive swelling during storage at 2°C; this is reflected in the increase in the fragility to hypotonic saline solutions. This property is not reversible by washing in isotonic saline. Red cells stored in sucrose-citrate solutions shrink. Erythrocytes in 5.4% dextrose-citrate

<sup>8</sup> DeGowin, E. L., Harris, J. E., and Plass, E. D., *J. Am. Med. Assn.*, 1940, **114**, 850.

<sup>9</sup> Jacobs, M. H., Glassman, N. H., and Parpart, A. K., *J. Cell. and Comp. Physiol.*, 1931, **60**, 95.

undergo rapid swelling which is due to the diffusion of dextrose and water into the cells. The process can be reversed by washing with isotonic saline. The addition of hypertonic dextrose solutions to blood-citrate produces hypertonic cell contents during storage. The transfusion of such cells results in intravascular hemolysis by action of the recipient's plasma. The osmotic activity of the erythrocytes thus becomes another criterion for the suitability of stored blood for transfusion. Blood collected in dextrose solutions should be chilled rapidly to avoid osmotic hemolysis during refrigeration.

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### Study with Radioactive Isotopes of Excretion of Calcium and Strontium by Way of the Bile.\*

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The significance of the bile as a pathway for the excretion of calcium has attracted attention because of the relatively high calcium concentration of gall bladder bile.<sup>1</sup> In experiments on 2 bile fistula dogs, Gillert<sup>2</sup> found that the calcium in the bile was about  $\frac{2}{3}$  the amount excreted in the urine.

Tracer studies with radioactive calcium and strontium offer a method of obtaining information on the excretion of these ions by way of the bile that simplifies many of the difficulties usually present in this type of experiment, such as the factor of reabsorption from the gall bladder<sup>3</sup> and the source of the calcium in the bile. Through the use of labeled ions, it is possible to determine the fraction of an administered dose of calcium or strontium that appears in the bile. The results of such experiments on bile fistula rats are described in this communication.

*Methods.* Rats weighing between 400 and 500 g, with an artificial gall bladder type fistula, prepared according to the operation of

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<sup>1</sup> Drury, D. R., *J. Exp. Med.*, 1924, **40**, 797.

<sup>2</sup> Gillert, E., *Z. ges. Exp. Med.*, 1924, **43**, 539.

<sup>3</sup> Johnston, C. G., Ravdin, I. S., Austin, J. H., and Morrison, J. L., *Am. J. Physiol.*, 1932, **99**, 648.