Biochemical Alterations During Storage of Human Blood from Male and Female Donors (35222)

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In the investigation of various conditions which might permit prolongation of the storage time of blood, little attention has been given to specific parameters related to the donor from whom the blood is obtained. One of these parameters which could possibly influence the preservation of the red cell during storage is sex. Male and female donors have different endocrine systems with formation of structurally different endogenous steroid hormones. Thus, the different circulating hormones in the bood could affect not only the metabolic but also the structural pattern of the red cell during preservation.

Previous studies (1) have shown that the uptake of progesterone by red blood cells (RBC) is very high and that when progesterone is added to stored human red blood cells the loss of osmotic resistance and the spontaneous hemolysis during storage are greatly minimized as compared to red cells stored without addition of progesterone (2, 3). It was, therefore, considered of interest to study osmotic fragility, spontaneous hemolysis, and other biochemical parameters in whole blood, from male and female donors, stored at 2° in acid–citrate–dextrose (ACD) solutions.

Materials and Methods. One unit of blood was obtained from each of 12 Caucasian healthy donors, six male and six females, 18–25 years of age. The female donors donated blood at 20–22 days after onset of menstruation. The blood was drawn into plastic bags containing 67.5 ml of ACD (National Institute of Health formula A)/450 ml of blood and was stored at 2°. Another sample of blood was obtained from a healthy female at the eighth month of pregnancy[1]: 50 ml of blood were drawn into a plastic bag containing 7.5 ml of ACD and the bag was also stored at 2°. At selected intervals during storage, aliquots of blood were removed, under sterile conditions, for analyses.

Adenosine triphosphate (ATP) was determined by the enzymatic test method of Hollaway (4) using the ATP kit of Boehringer Mannheim Corporation (New York, N.Y.).

Total hemoglobin in blood, hemoglobin released in plasma, and percentage hematocrit were assayed according to procedures previously described (2).

Osmotic fragility was determined on erythrocytes prepared from the aliquots of blood removed at the indicated time of storage, using test tubes with several NaCl concentrations as previously reported (2).

Results. Blood was drawn from female donors at a time, in the menstrual cycle, when the concentration of progesterone is at its highest level. An indication of the progesterone secretion was obtained by the assay of urinary pregnanediol (5), chief excretion product of progesterone. At 8–10 days and at 20–22 days after onset of menstruation, values of urinary pregnanediol for 24-hr samples were 1 to 2 mg and 4 to 5 mg, respectively, demonstrating a higher excretion of this steroid during the luteal phase, in agreement with the data reported by Klopper (5).

Changes of pH and ATP levels during storage of blood obtained from male and female donors[2] are shown in Fig. 1. Initially the pH of male and female blood shows essentially the same value, but with prolonged time of

[1] The authors are indebted to Dr. S. D. Litwin and Mrs. D. M. Guthrie of the Blood Transfusion Division of our laboratory for being instrumental in procuring this sample of blood at the Ireland Army Hospital, Fort Knox, Kentucky.

[2] Blood from male and female donors is referred to also as "male" or "female" blood for brevity and convenience.

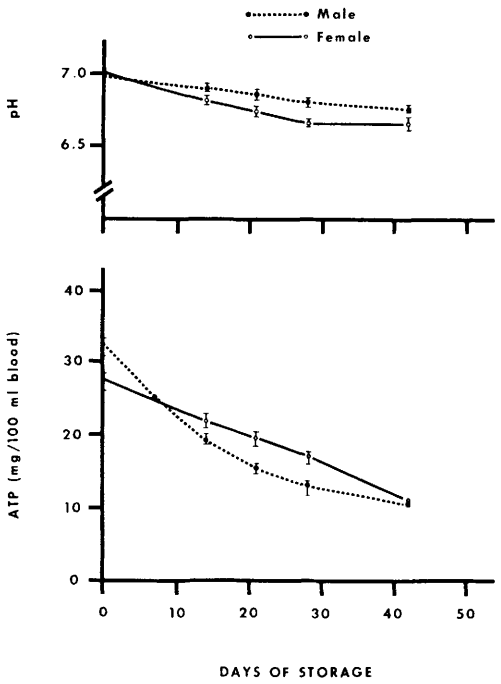

FIG. 1. Change in pH and ATP levels occurring in human blood, obtained from male and female donors, at different times of storage at 2° in acid–citrate–dextrose solutions. The intersections on the ordinates indicate experimentally determined values at the times of collection of blood. Means and ranges of values obtained from duplicate determinations on blood from six male and six female donors are represented. The differences between ATP levels of male and female blood at 0, 21, and 28 days are significant ($p<0.01$).

storage the decrease of pH in the female blood becomes progressively greater than that observed in the male blood. Greater differences are observed in the changes of ATP levels in male and female blood during storage. At the time of collection of blood (time 0), invariably, the ATP level of the female is lower than that of the male blood. In a number of such determinations in our laboratory, the ATP level in the female blood at time 0 is 12–18% lower than that observed in the male blood. As the time of storage is prolonged, the male blood shows a sharp drop in ATP value during the first 14 days of storage; the decrease of ATP continues afterwards but at a slower rate and, at 42 days of storage, ATP has reached a value of 32.3% of that at time 0. In the female blood a constant rate of decrease is observed during the storage period; at 42 days of storage the ATP level is 40% of that at time 0. It seems that the conservation of ATP is better maintained in the female blood than in the male blood.

The amount of hemoglobin released in the plasma is shown in Table I for male and female blood. As the time of storage is prolonged, the percentage hemoglobin determined in the plasma increases for both male and female blood; however, for male blood the increase is much greater and at 42 days of storage the plasma hemoglobin value is more than twice that observed in the female blood.

Statistical t test analysis of the differences obtained for blood of male and female donors show that these differences in ATP levels at 0, 21, and 28 days of storage (Fig. 1) as well as in the plasma hemoglobin values at 14, 21, 28, and 42 days of storage (Table I) are significant beyond 0.01 and 0.001 level of significance.

Osmotic fragility curves of red cells from blood of male and female donors at 0 time, 28 and 42 days of storage are shown in Fig. 2. Erythrocytes prepared from fresh blood show the same osmotic fragility whether they are obtained from blood of male or female donors. After 28 days of storage marked differences are observed. At this time, considering a NaCl concentration of 0.6%, erythro-

TABLE I. Hemoglobin Released in the Plasma at Different Time of Storage Using Blood from Male and Female Donors.

Days of storage	Hb in plasma[a] (mg/100 ml; means and ranges)[b]	
	Male	Female
0	6.0 (4.5– 6.9)	4.5 (4.0– 5.0)
14	33.8 (32.0– 35.1)	11.0 (9.6– 15.8)
21	63.0 (52.0– 70.1)	25.6 (22.5– 27.5)
28	132.4 (120.1–142.1)	69.7 (66.7– 72.3)
42	425.2 (411.2–434.4)	206.3 (195.9–214.8)

[a] For male and female blood, the plasma hemoglobin values at 14, 21, 28, and 42 days of storage are significantly different ($p<0.01$).

[b] From duplicate determinations on blood from six male and six female donors.

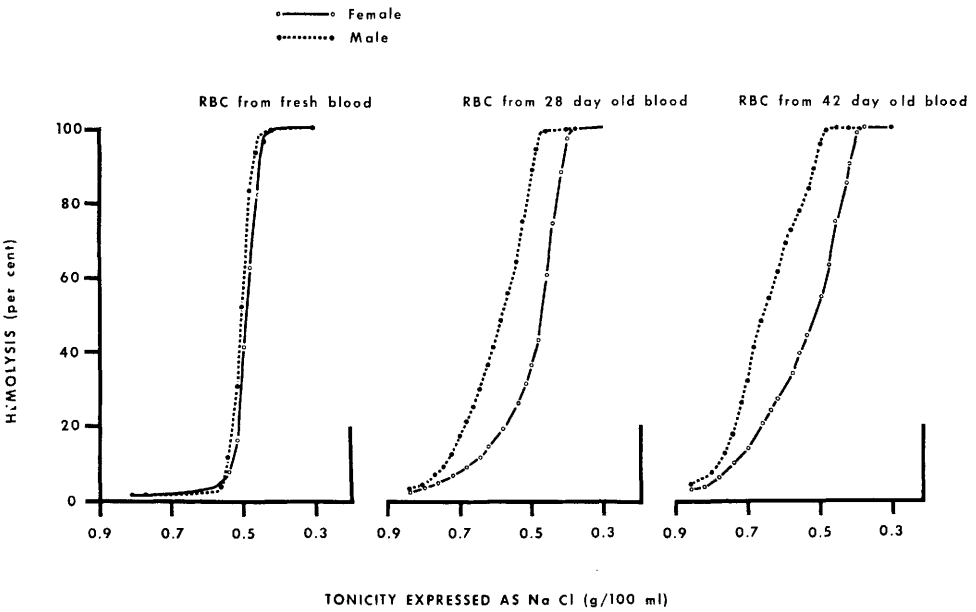

FIG. 2. Osmotic fragility curves of red cells from blood of male and female donors at different times of storage at 2° in acid–citrate–dextrose solution.

cytes from female donors show 17% hemolysis; whereas erythrocytes from male donors demonstrate about 41% hemolysis. As the time of storage is prolonged, this difference becomes significantly greater, and, at 42 days of storage, at 0.6% NaCl concentration, the percentage hemolysis is 30 and 69% for red cells prepared from blood of female and male donors, respectively. The fragility curves in Fig. 2 are typical for blood from male and female donors. Similar patterns have been obtained with several different male and female blood units.

The results obtained with blood from an 8-month pregnant female follow the same pattern observed with blood drawn from female donors at 20–22 days after onset of menstruation. The values for osmotic fragility, hemoglobin released in the plasma, and ATP levels are essentially the same as those for normal female donors but differ markedly from those obtained for blood of male donors.

Discussion. The results shown in Fig. 2 and Table I clearly demonstrate that by the criteria of osmotic fragility and hemoglobin released in the plasma, the blood obtained from female donors is able to withstand the conditions of stress of prolonged storage better than the blood from male donors. A possible explanation for these differences could be found in the structurally and pharmacologically different endogenous steroid hormones formed by the endocrine systems in the male and female.

As previously reported (2, 3), progesterone added to red blood cells minimizes the storage lesions so that in erythrocytes preserved with progesterone, osmotic resistance is greatly increased and spontaneous hemolysis markedly reduced when compared with erythrocytes preserved without addition of progesterone. It has also been demonstrated (1) that progesterone interacts with rat blood cells; and recent results in our laboratory (6) have shown that a strong binding exists between progesterone and certain components of the human red blood cell membrane. The presence of progesterone circulating in the female donors could be responsible for maintaining osmotic resistance and for limiting the spontaneous hemolysis of the red cells *in vitro* during storage. As the red cell deteriorates during storage, a breakdown in osmotic regulation occurs which is thought to result from increased membrane permeability. It is tempting to speculate that the steroid hormones in the female blood minimize this in-

crease in membrane permeability, limiting or preventing the deterioration of the structure of the cell membrane by interacting with components of this membrane. If the structural integrity of the membrane is maintained, the metabolism occurring in the cell would follow a more normal pattern. This is evidenced by a linear decline and better maintenance of ATP in the red cells from female donors than that observed in those from male donors, as shown in Fig. 1. Since the supply of this high energy phosphate is available at higher levels, the glycolytic process would continue at a more normal rate and the consumption of glucose and formation of lactic acid would explain the slightly lower values of pH in the blood from female as compared to those of the blood from male donors (Fig. 1).

The results showing that the erythrocytes from female donors have greater osmotic resistance than those from male donors (Fig. 2) could be explained by the affinity of female hormones for components of the red cell membrane. As mentioned before, an interaction between progesterone and cell membrane could minimize an increase in the membrane permeability and thus limit the deterioration of the cell during storage. This possibility is supported by the results in Table I, which demonstrate that, during storage, the percentage hemoglobin released in the plasma is much lower for erythrocytes obtained from blood of female donors than for those prepared from blood of male donors.

These differences between male and female blood have been observed in an *in vitro* study. It would be of great interest to follow these investigations with post-transfusion survival studies since Gibson (7) has reported that a rough correlation exists between the osmotic resistance and post-transfusion survival of the red cells.

Summary. Units of human blood were obtained from male and female donors and compared for changes in pH, ATP levels, plasma hemoglobin, and osmotic resistance during storage in ACD for a period up to 42 days. The data show that, by the criteria of the above *in vitro* determinations, the blood obtained from female donors is able to survive the storage lesions better than the blood from male donors. These results are attributed to the different endocrine systems in the human male and female and the capacity to form structurally and pharmacologically different endogenous steroid hormones. The possibility that these steroid hormones could interact with components of the red cell membrane and limit an increase in cell membrane permeability is discussed. A sample of blood obtained from an 8-month pregnant human female was also studied, the pattern of preservation of such blood was essentially the same as that observed with the blood of female donors.

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