Osmotic Resistance and Post-Transfusion Survival of Human Erythrocytes Stored in the Presence of α-Tocopheryl Disodium Phosphate. (24283)

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Human erythrocytes undergo a progressive decrease in osmotic resistance during storage at 4° and this effect has been employed as an *in vitro* measure of the state of preservation of blood(1-2). During the course of a screening program for new blood preservatives it was observed that tocopheryl phosphate salts, over a narrow concentration range, markedly retarded this decrease in osmotic resistance. The effect of tocopheryl phosphate salts on post-transfusion survival of red cells is here described.

Methods. Osmotic resistance was measured at 0.6% buffered saline by the procedure of Parpart *et al.*(1.) Manner of preparation and handling of samples was essentially that of Schales(2). Phosphate partitions were determined on whole blood by the method of Umbreit, Burris, and Stauffer(3).

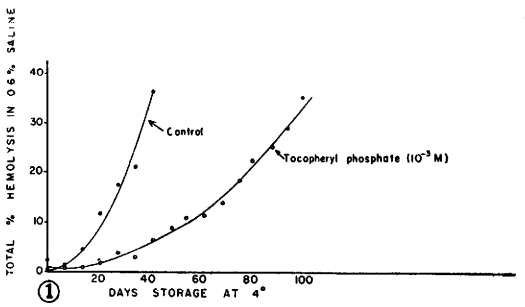
Post-transfusion survival was determined in normal human volunteers using Cr^{51} labeled red cells as reported previously(4). One hundred ml of blood was withdrawn by gravity from each subject into sterile bottles containing either anticoagulant acid citrate solution B. USP XV (ACD) alone or ACD plus disodium α-tocopheryl phosphate to a final molarity of 10^{-3}. The blood was then refrigerated at a constant temperature of 4°C for varying periods of time. Prior to reinjection the blood was labeled with 150 μc of Cr^{51}, allowed to stand at room temperature for one hour, then the red cells were washed 3 times with cold physiologic saline. Fifty ml of the washed, Cr^{51}-labeled erythrocytes were then injected into the same donors. Counts/min/ml of red blood cells at 15 min after injection were used as 100% activity. Samples of blood were withdrawn at appropriate intervals beginning 24 hours after injection. Survival was calculated by the formula,

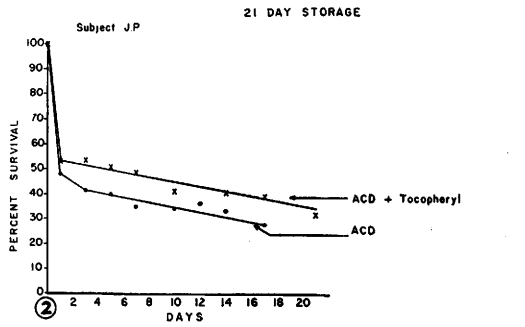
$$\% \text{ survival} = \frac{\text{c./min./ml sample x 100}}{\text{c./min./ml 100\% sample}}.$$

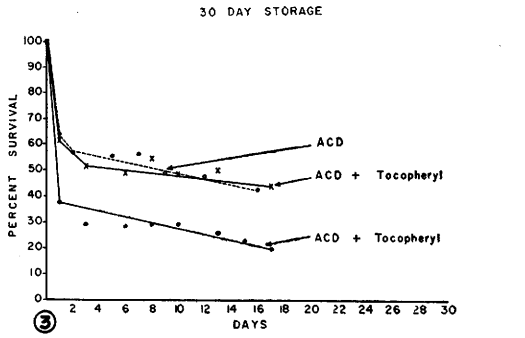
Results. The effect of storing ACD blood in the presence of 10^{-3}M disodium α-tocopheryl phosphate is demonstrated in Fig. 1. The total % hemolysis at 0.6% buffered saline after 100 days of storage was slightly less than that obtained in the controls after 42 days of storage, indicating marked "protection." The effect of varying the concentration of tocopheryl phosphate was also determined (Table I). Concentrations of 2 x 10^{-3}M were quite hemolytic, while concentrations of 10^{-4}M and less had no detectable effect. The "protective effect" was obtained at a level just below that inducing extensive hemolysis. Preliminary results with the sodium salt of tocopheryl succinate (Table I) gave results comparable to those obtained with the phosphate ester indicating the general nature of the effect.

Addition of tocopheryl phosphate to the solutions employed in the osmotic resistance test at concentrations comparable to those obtained above, were without significant effect. Therefore, storage of ACD-blood in the presence of tocopheryl ester salts is required to obtain this effect on osmotic resistance.

It then became desirable to determine degree of preservation by actual post-transfusion measurements. Since α-tocopheryl phosphate is known to have anti-thrombic activity (5-6), it was first necessary to determine the effect of infusions of this compound on the coagulation system. Intravenous injection of 2.5 mg of disodium α-tocopheryl phosphate/kg body weight in the dog (rapid infusion), and 1.25 mg/kg body weight in man (slow infusion in 5% dextrose over 25 min) caused no significant alteration in bleeding time, coagulation time, platelet count, prothrombin time,







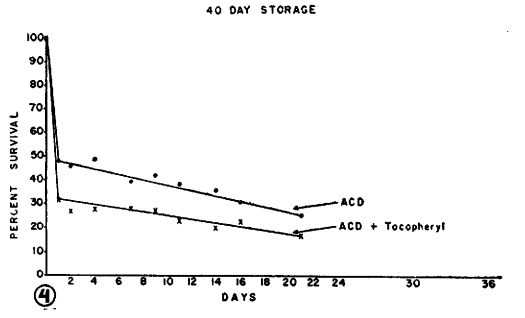


FIG. 1. Effect on osmotic resistance of storing human ACD blood at 4°C in presence of 10^{-3}M disodium dl-α-tocopheryl phosphate.

FIG. 2. Post-transfusion survival of human erythrocytes stored 21 days at 4°C.

FIG. 3. Post-transfusion survival of human erythrocytes stored 30 days at 4°C.

FIG. 4. Post-transfusion survival of human erythrocytes stored 40 days at 4°C.

thrombin coagulation time or recalcification time. Dog blood was then stored in ACD plus α-tocopheryl phosphate for 21 and 42 days at 4°, and reinfused into the donor dogs without toxic effects.*

Results of post-transfusion survival studies in humans† are presented in Fig. 2, 3, and 4. It is obvious that no enhancement of erythrocyte survival occurs when ACD-blood is stored in the presence of α-tocopheryl phosphate. Phosphate partition determinations (Table II), completed a few days before infusion, demonstrate that the energy-rich organic phosphate ester stores were equally depleted in the presence and absence of α-tocopheryl phosphate.

Discussion. The effects obtained with tocopheryl esters appear to parallel those obtained by Chaplin, Crawford, Cutbush, and Mollison(7) with a phenothiazine derivative (R.P. 3300) related to Phenergan. Both (a) reduce rate of decrease in osmotic resistance of stored blood; (b) are hemolytic at concentrations only slightly higher than required to give "protection"; (c) are surface-active in nature(7-8); (d) do not improve post-transfusion survival; (e) exhibit enhanced lytic effect on removal of serum proteins. Addition of 10^{-3}M α-tocopheryl phosphate to sheep blood did not cause hemolysis while addition to washed cells suspended in saline caused rapid, extensive, hemolysis. Therefore, the increased osmotic resistance of erythrocytes from blood stored in the presence of α-tocopheryl ester salts may be another example of the anomalous behavior of erythrocytes in the presence of sub-hemolytic amounts of hemolytic compounds.

Summary. Presence of 10^{-3}M disodium α-tocopheryl phosphate in human ACD-blood greatly retards rate of decrease in osmotic resistance of the erythrocytes that occurs on

* In this species the use of 10^{-3}M α-tocopheryl phosphate was detrimental as measured by spontaneous hemolysis and osmotic resistance.

† The clotting mechanism of these subjects was tested before and 15 min. after injection of the blood. No changes in bleeding, clotting, recalcification, prothrombin, prothrombin consumption, or thrombin times were noted.

TABLE I. Effects of Addition of Tocopheryl Ester Salts to Human ACD Blood on Erythrocyte Osmotic Resistance during Storage.

Compound added	Additive final molarity	% buffered NaCl in hemolysis test	Days blood stored at 4°C: 13	24	36	45	58	69	84	99
			% hemolysis							
Disodium d,l-α-tocopheryl phosphate	2×10^{-3}	.6	10.7	24.6	40.4					
	"	1.0	8.5	16.5	32.3					
	10^{-3}	.6	3.5	3.8	5.6	7.6	11.3	16.1	18.2	26.4
	"	1.0	1.9	2.8	3.4	4.3	5.1	6.0	9.0	12.6
	10^{-4}	.6	7.7	7.8	14.3	12.1	39.8			
	"	1.0	1.0	1.9	3.9	3.8	5.2			
d-α-tocopheryl succinate	10^{-3}	.6	1.5	3.8	4.3	4.9	6.9	8.8	10.2	15.7
	"	1.0	1.7	1.7	4.3	4.4	3.7	4.9	8.5	10.2
	None	.6	3.3	5.3	10.0	16.6	33.3	52.1		
	"	1.0	1.5	2.0	2.8	4.3	4.7	9.3		

TABLE II. Changes in Phosphate Partition of ACD Blood* on Storage at 4°C in Presence of 10^{-3}M α-Tocopheryl Phosphate.

Days blood stored at 4°	No. of subjects	Additive	% of total phosphorus: Inorganic	Easily hydrolyzable	Difficulty hydrolyzable	Non-hydrolyzable
0	8	None	11.4	18.4	10.0	60.2
18	2	Tocopheryl	74.4	9.5	4.8	11.3
24	2	"	77.7	6.9	4.3	11.2
24	1	None	74.1	8.9	4.5	12.5
36	1	Tocopheryl	83.4	4.0	2.5	10.1
36	1	None	77.9	7.7	4.0	10.4

* These human bloods were subsequently employed in post-transfusion survival experiments reported in Fig. 2, 3, and 4.

storage at 4°C. Post-transfusion survival of erythrocytes from blood stored in this manner is no better than that of erythrocytes from blood stored in ACD alone. It is emphasized that enhanced osmotic resistance of preserved blood is not necessarily related to enhanced post-transfusion survival.

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