SEQUENTIAL CHANGES IN COAGULATION FACTORS AND

CELLULAR COMPONENTS OF ACD-STORED BLOOD.

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ABSTRACT.

Sequential changes in blood cell components and coagulation factors during short-term storage in blood bank condition was studied in 20 blood samples stored at 4°C in ACD solution.   The recovery rates of leukocytes, leukocyte viability and platelets were comparable to that observed by others in both ACD and CPD blood.   The recovery rate of leukocytes of 85.45 $\pm$ 18.30% at one week and 80% at 10 days of storage were similar to that of the CPD blood.   Most of the leukocytes disintegrated were PMNs.   The finding of higher recovery rate of platelets (46% at 2 weeks of storage) in our ACD blood is probably due to the fact that spontaneous clumping of platelets occurred less rapidly in ACD blood. Factor VIII activity dropped rapidly within the first few hours and only 33.90 $\pm$ 8.68% of its activity was detected during the first day of blood collection.   Factor V activity decreased more gradually to only 40% of the original activity by day 7.   Factor VII activity increased gradually within the first week of storage due to the cold-promoted enhancement of its activity via plasma kallikrein and contact activation.

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INTRODUCTION.

Sodium citrate was first introduced as a useful anticoagulant for blood collection and transfusion by Lewisohn in 1915[1]. The addition of dextrose to this anticoagulant further extended the usable time for the collected blood[2]. The replacement of some citrate with citric acid by Loutit et al in 1943[3] resulting in a solution almost identical to the present Acid-citrate-Dextrose (ACD) solutions. Although other anticoagulants, such as heparin and chelating agents, have been used for special purposes, ACD has remained the standard blood preservative for many years. The ACD preserved blood has a usable span of only 21 days before decreasing red cell viability made it unsatisfactory for transfusion purposes. In 1957, Gibson et al[4] introduced citrate-phosphate-dextrose (CPD) solution which has a higher pH, lower citrate concentration and a more isotonic environment than ACD solutions. Results of several studies indicated that the percentage of red cell surviving at 28 days of storage in CPD was significantly greater than in ACD solutions. In additions, the presence of sodium phosphate in CPD solution results in better conservation of 2.3 DPG than occurs in ACD stored blood and thus maintains better oxygen transport efficiency.

Although CPD is considered to be the best routine blood preservative which should prolong the usable span of stored red cells, a few limitations to its widespread use exist. Prolongation of the storage time by CPD results in higher plasma levels of ammonia, potassium and free hemo-globin (5-7) and greater amount of intracellular potassium liberated from stored red cells that are lysed in the immediate posttransfusion period[7]. The concentration of plasma sodium is greater in CPD stored blood due to the use of sodium citrate and sodium phosphate CPD solution[5]. These substances could result in potentially deleterious effects in patients with hepatic and renal diseases. In addition, the formation of microaggregates of leukocytes and platelets is found to be greater in CPD than in ACD stored blood[8] which is probably due to the higher pH of the former[9].

In practice, blood is stored in ACD solution and the storage time is rarely longer than 2 weeks in our blood bank. We are reporting the sequential changes in coagulation factors, chemical and blood cell components occurred in ACD stored blood in such short-term storage.

MATERIALS AND METHODS.

Blood samples were collected from 20 healthy male adults according

to the guidelines of the American Association of Blood Bank[10]. Approximately 400 ml of blood were collected into 10 plastic bags and 10 glass bottles containing 67 ml and 100 ml of ACD solution respectively. After thorough mixing, whole blood samples were aliquoted and stored at $4°C$ in sterile plastic or glass containers accordingly. Each blood samples were analized immediately following collection and on days 1, 2, 3, 4, 5, 6, 9 and 13. White blood cell count, differential leukocyte count, leukocyte viability test (trypan blue dye exclusion test) and platelet count were determined on all 20 blood samples. Plasma concentrations of sodium and potassium were determined by atomic absorption spectroscopic technique [11]. Plasma levels of coagulation factors V, VII, VIII, IX, X and XII were determined [12,17] on 10 blood samples collected and stored in plastic containers.

RESULTS

The baseline values of the blood cell components, coagulation factors and their sequential changes during storage at $4°C$ were summarized and shown in Table 1 and Figure 1. The recovery rate of leukocytes of 85.45 $\pm$ 18.30% at one week was slightly low but the rate of 80% at 10 days of storage was comparable to that of

the CPD blood [18]. Most of the leukocytes disintegrated were PMNs. At 2 weeks 66.0 $\pm$ 3.13 % of the remaining leukocytes were still viable, and the recovery rate of platelets was 46%. The plasma K^+ concentration rise gradually from the baseline value of 3.30 $\pm$ 0.8 mEq/L to 15.5 $\pm$ 2.95 mEq/L after 2 weeks of storage comparable to that observed by others for both ACD and CPD blood [7, 19]. Significantly low levels of factor VIII (33.90 $\pm$ 8.68%), IX (36.5 $\pm$ 5.08%) and XII (26.4 $\pm$ 7.59%) were observed in the first day of blood collection. Factor V activity decreased gradually to 40% of the original activity by day 7. Factor VII activity increased 3 folds during the first week of storage.

COMMENTS.

The plasma K^+ concentration of our ACD blood increased in direct proportion to the storage time (Figure 1). The magnitude of the increment was comparable to that observed by others for both ACD and CPD blood [5-7, 20-24]. Normally red cells contain approximately 100 mEq K^+/ L of packed red cells and plasma contains only 3-5 mEq K^+/L. Stored red cells have been known to lose K^+ into the surrounding plasma [25] resulting in increased plasma K^+ concentration and decreased K^+ content of red cells.

The increased in plasma K^+, LDH and free hemoglobin levels reflect either red cell hemolysis or alteration in red cell membrane permeability [5]. Nakao et al [26] and Haradin et al [27] have demonstrated a progressive alteration in the shape of the stored red cells from a biconcave disc when fresh to a microspherocyte after 8 weeks. The progressive loss in total erythrocyte lipids accompanying in vitro storage in ACD which approximates 20 to 30% as 6 weeks [27] had been shown to be associated with losses of portions of the red cell membrane varying in size from macromolecular aggregates to microscopically visible buds [28, 29]. Recent evidences indicate that the plasma K^+

concentration increases in direct proportion to the storage time [25] and independent to small degree of hemolysis that occur during storage [7]. At 4°C the rate of consumption of dextrose is approximately 0.05 mMoles/L. of red cells/hours [30, 31] which is at least 30 times less than at 37°C and the active transport of Na^+ and K^+ is almost halted. The increment plasma K^+ concentration represents K^+ that leaked from intact red cells, and the rate of efflux is inversely related to the ATP content of the stored red cells [32].

It is now realized that the deterioration of red cells during storage is a different process from aging in vivo. The ATP content of stored red

Table 1. Sequential Changes of Cellular Components of ACD Blood

Storage Time (days)	TOTAL WBC	WBC VIABILITY %	PMNs %	PLATELETS x 10^3 / cu.mm.
0	11,925 ± 2,266	100	57.0 ± 7.17	309.0 ± 60.78
1	11,795 ± 3,265	99.7 ± 0.48	70.0 ± 7.12	289.8 ± 68.32
2	11,215 ± 2,412	96.9 ± 1.20	58.4 ± 9.96	272.2 ± 68.02
3	10,760 ± 2,496	91.8 ± 1.93	59.1 ± 13.67	252.1 ± 52.96
4	9,650 ± 2,158	88.6 ± 2.80	55.7 ± 10.27	243.8 ± 44.96
5	10,894 ± 2.282	84.5 ± 2.37	53.3 ± 13.90	224.6 ± 33.18
6	10,190 ± 2,183	81.0 ± 5.14	50.2 ± 18.15	226.5 ± 50.25
9	11,225 ± 3,464	72.1 ± 2.56	47.7 ± 17.67	205.6 ± 48.51
13	7,915 ± 1.720	66.0 ± 3.13	37.6 ± 14.66	143.1 ± 50.86

cells diminishes with time [25] but it undergoes little change while red cells age in vivo [33]. Red cells loss their ability to survive in vivo before they hemolyzed in vitro. The damages in vitro which lead to loss of viability of red cells are not well understood. Factors which affect the osmotic fragility of stored red cells do not have any influence on their viability and substances which retard the rate

of hemolysis of stored blood do not necessarily prolonged the viability of red cells [34]. CPD solution is superior to ACD solution because it is more isotonic, better conservation of 2, 3, DPG and pCO_2, maintains higher pH throughout storage with significantly lower plasma K^+ and free hempglobin levels (4, 5) which should prolong the usable span of stored red cells. But there was litfle
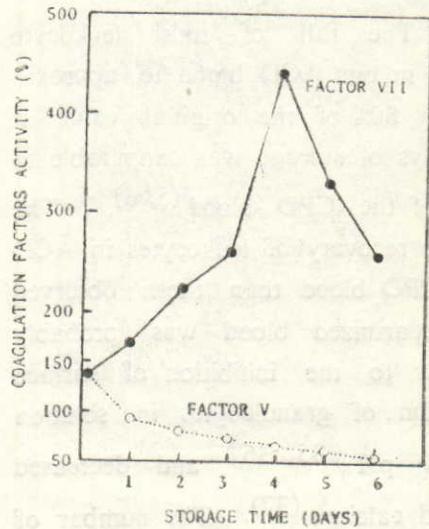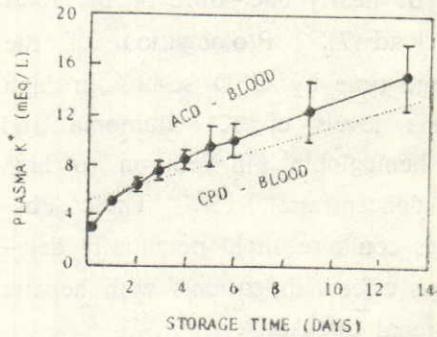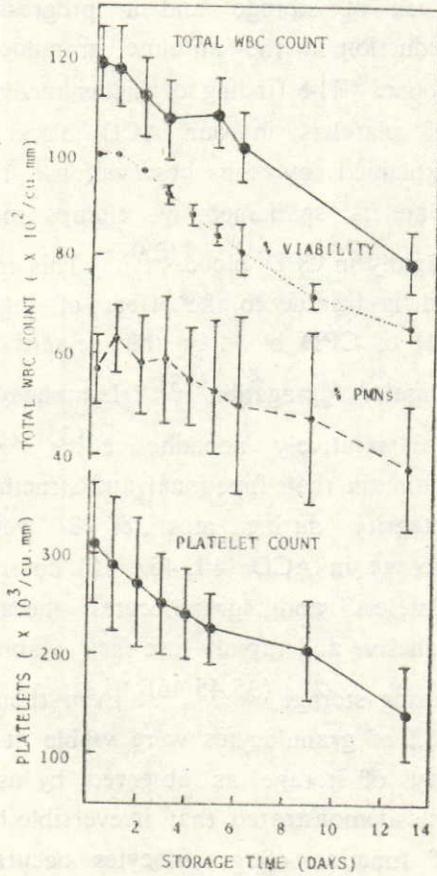

FIGURE I: The sequential changes of leukocytes, platelets, plasma potassium level and coagulation factors of ACD blood during storage.

difference in red cell survival when blood was stored in these two solution up to 21 days [5]. When blood was preserved for 2 to 3 weeks approximately 15 to 25% of transfused red cells were destroyed in vivo in the first 24 hours after transfusion [7]. Thus the total K^+ load from a transfusion can not be evaluated from plasma values alone. The red cell lysis in vivo must also be considered since its contribution may be nearly one-third of the total K^+ load (7). Prolongation of the storage time by CPD sesults in high plasma levels of K^+ ammonia and free hemoglobin (in addition to high Na^+ concentration) (5). These substances could result in potentially deleterious effects in patients with hepatic and renal diseases.

The fall of total leukocyte count in our ACD blood to approximately 80% of the original value at 10 days of storage was canparable to that of the CPD blood [5, 6]. The higher recovery of leukocytes in ACD and CPD blood than those observed in heparinized blood was probably related to the inhibition of surface adhesion of granulocytes in solution of low pH [35, 36] and decreased ionized calcium [37]. The numbar of platelets in our ACD blood decreased to 61% by day 10 and to 46% at 2 weeks was slightly higher than those

observed in CPD blood [5]. The decreased numher of platelets and leukocytes observed is explained by the fact that they form clumps during storage [38-41]. Light and electron microscopie examinations revealed that the microaggregates which developed in stored blood consist of degenerated platelets and leukocytes [40-42]. Coincident with this there was a drop in the platelet count during the first week of storage and a progressive reduction in the absolute granulocyte count. The finding of higher recovery of platelets in our ACD blood is explained by the observations that platelets spantaneously clumps more rapidly in CPD blood [8,9]. This may partly be due to the effect of higher pH of CPD blood on the velocity of platelet aggregation [43]. Lymphocytes are relatively nonadhesive [35] and maintain their functional and structural integrity during most of 3 weeks storage in ACD (44, 45). In contrast, platelets and granulocytes became adhesive and rapidly lose their viability during storage [35,45,46]. Even though 66% of granulocytes were viable at 14 days of storage as observed by us it was demonstrated that irreversible loss of function of granulocytes occurred after a few hours and granulocyte mobility, phagocytosis and O_2 consumption were markedly diminished

after one week of storage in ACD plasma [18, 47]. More recent observations indicated that granulocytes in whole blood stored for 24 hours retaine their phagocytic and bactericidal activities [18, 45, 48]. The decline in function after 24 hour of storage further indicated that the granulocyted would have only limited value for transfusion therapy thereafter. Platelets lose their ability to survive in vivo very rapidly and whole blood stored in ACD even 24 hours is a poor source of viable platelets [49, 51]. It takes only 24 hour storage for the percentage of viable platelets to fall to 5% level [22].

Factor VIII activity dropped rapidly during storage at $4°C$ [52-54]. Our baseline factor VIII activity was very low due to the dilution effect of large amount of ACD solution and delayed factor VIII determination. At 24 hours of collection the factor VIII activity was approximately 32% which is comparable to those observed by others [54, 55]. Our results confirmed the instability of factor VIII and supported the recommendation that cryoprecipitate preparation should be processed within 4 hours of collection. The other coagulation factors known to deteriorate appreciably on storage is factor V. Factor V activity decreased rapidly to 40% of the initial concentration at day 7 comparable to

those observed by others (5, 55-57). Factor VII activity increased during storage similar to that observed earlier [55]. This phenomenon is explained on the fact that cold - promoted enhancement of factor VII activity occurred by surface contact via factor XII and plasma kallikrein [58-62].

บทคัดย่อ

คณะผู้รายงานได้ศึกษาการเปลี่ยนแปลง ของส่วนประกอบต่างๆของเลือดจากคนธรรมดา จำนวน 20 ราย ที่เก็บไว้ใน ACD solution ที่ 4°C ในธนาคารเลือด ผลการศึกษาแสดงว่า เลือดที่เก็บไว้ใน ACD ในระยะสั้นคือประมาณ 2 สัปดาห์ มีคุณภาพเท่าเทียมกับที่เก็บไว้ใน CPD solution หลัง 24 ชั่วโมงแรก PMNs จะแตกทำลายไป ทำให้จำนวนของเม็ดเลือด ขาวลดลงตามลำดับ เหลือ85.45 ± 18.30% ในสัปดาห์แรก และเหลือ 80% ในวันที่ 10 จำนวนของเกล็ดเลือดลดลงตามลำดับ เช่นเดียว กัน จนเหลือเพียง 46% ของค่าเดิมใน 2 สัปดาห์ ค่าที่ได้นี้สูงกว่าเลือดที่เก็บไว้ใน CPD เนื่อง จากเกล็ดเลือดเกาะกลุ่มกันน้อยกว่าที่เกิดขึ้นใน CPD ระดับของ factor VIII ลดลงอย่างรวดเร็ว ภายในเวลาไม่กี่ชั่วโมง ทำให้มีระดับเพียง 40% ภายในวันแรก แต่วันต่อไปก็มีระดับค่อนข้าง คงที่ ระดับ factor V ลดลงช้าๆจนเหลือเพียง 40% ของค่าเดิมในวันที่ 7 ระดับ coagulation factor อื่นๆค่อนข้างคงที่ แต่ค่าของ factor VII กลับเพิ่มสูงขึ้นหลายเท่าตัว ภายในสัปดาห์ แรก ทั้งนี้เป็นเพราะในขณะที่เก็บไว้ในความ เย็น ฤทธิ์ของ factor VII จะถูกกระตุ้นด้วย surface contact ผ่านทางระบบ Kallikrein.

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