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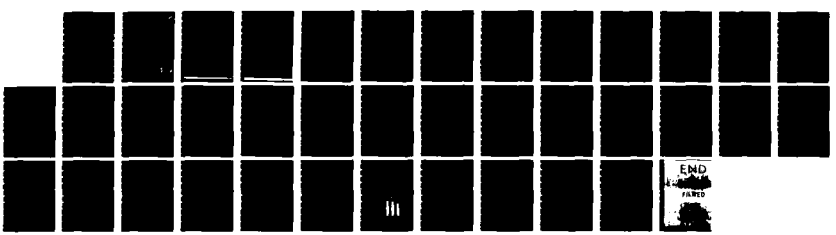

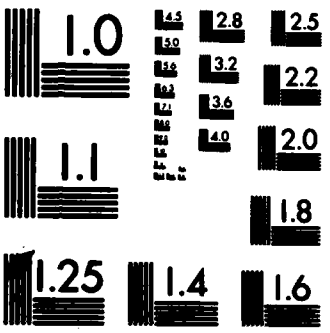

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Viability of stored rabbit erythrocytes carrying no C3c

ANNUAL REPORT

Irma O. Szymanski, M.D.

January 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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University of Massachusetts Medical Center
Worcester, Massachusetts 01605

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The goal of this study was to evaluate whether the third component of C3, bound to RBC during liquid storage, contributes to the preservation injury. Rabbit model was used for these studies. Thus, studies were performed to validate the use of rabbit model in RBC preservation studies.

It was shown that rabbit RBC undergo similar changes during liquid storage in CPD plasma as human RBC. The concentration of ATP in fresh rabbit RBC was higher than in human RBC, while the rate of decline at 4C was more rapid in

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rabbit RBC. The concentration of 2,3 DPG was about two times higher in fresh rabbit RBC than in fresh human RBC and the rate of decline of this metabolite at 4C was similar in rabbit and human RBC. Rabbit RBC stored at 4C in CPD plasma were shown to take up C3 (the third component of complement) during storage. The viability of rabbit RBC after various periods of storage at 4C was very similar to that of human RBC, indicating that rabbit RBC developed the storage lesion at 4C in CPD plasma at about the same rate as human RBC. Furthermore, it was shown that the measurement of 24 hour survival is an acceptable way of quantitating the storage lesion in rabbit RBC since damaged RBC are removed rapidly within 24 hours and the the remaining RBC have a normal long-term survival in the absence of immunological incompatibility. It appears therefore, that the rabbit model is an easy, inexpensive way to evaluate the effectiveness of new preservation technics and the effect of other variables such as transportation, on RBC preservation. The rabbit model was also deemed suitable for basic research studies on RBC preservation.

In evaluating the role of RBC-bound C3 on the preservation injury, we stored RBC in heated plasma (60C for two hours) that prevented C3 uptake by RBC during storage. The viability of such RBC was no better than that of RBC to which C3 was attached. It was thought that the storage environment in heated plasma was damaging RBC. An alternative approach of storage in Adsol solution is suggested. This method of preservation permits maintenance of RBC ATP but does not support C3 binding by RBC.

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SUMMARY

The goal of this study was to evaluate whether the third component of complement, C3, that binds onto RBC during liquid storage, contributes to the preservation injury. Rabbit model was used in these studies. Thus, studies were also performed to validate the use of rabbit model in RBC preservation studies.

It was shown that rabbit RBC undergo similar changes during liquid storage in CPD plasma as human RBC. The concentration of ATP in fresh rabbit RBC was higher than in human RBC while the rate of decline at 4C was more rapid in rabbit RBC. The concentration of 2,3 DPG was about two times higher in fresh rabbit RBC than in fresh human RBC and the rate of decline of this metabolite at 4C was similar in rabbit and human RBC. Rabbit RBC stored at 4C in CPD plasma were shown to take up C3 (the third component of complement) during storage. The viability of rabbit RBC after various periods of storage at 4C was very similar to that of human RBC, indicating that rabbit RBC developed the storage lesion at 4C in CPD plasma at about the same rate as human RBC. Furthermore, it was shown that the measurement of 24 hour survival is an acceptable way of quantitating the storage lesion in rabbit RBC since damaged RBC are removed rapidly within 24 hours and the remaining RBC have a normal long-term survival in the absence of immunological incompatibility. It appears, therefore, that the rabbit model is an easy, inexpensive way to evaluate the effectiveness of new preservation technics and the effect of other variables such as transportation, on RBC preservation. The rabbit model was also deemed suitable for basic research studies on RBC preservation.

In studies evaluating the role of RBC-bound C3 on the preservation injury, we stored RBC in heated plasma (60C for two hours). This prevented C3 uptake by RBC during storage. The viability of such RBC was no better than that of RBC to which C3 was attached. It was thought that the storage environment in heated plasma was damaging RBC. An alternative approach of storage in Adsol solution is suggested. This method of preservation permits maintenance of RBC ATP level but does not support C3 binding by RBC, while RBC are not exposed to heated plasma so that we can determine more clearly whether C3 plays a role in development of preservation injury.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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Statement of the Problem

The studies reported here were carried out in order to determine whether C3 bound to RBC membrane during storage at 4C has a role in inducing "preservation injury", i.e., loss of RBC viability.

A rabbit model was utilized for these studies. It was necessary to validate the rabbit model for studies of RBC preservation.

Background

During storage of blood at 4C there is a gradual deterioration of RBC viability(1) and oxygen delivery function(2). The longer the blood is stored, the larger the fraction of nonviable RBC becomes(3). Thus, the loss of RBC viability poses the ultimate limit to the length of time that RBC can be stored at 4C.

Approaches to lengthening the useful storage period beyond the standard limit of 21 days are based on procedures that favor resynthesis of RBC ATP during storage. To this end, adenine and extra glucose are added to CPD anticoagulant solution(4-8). This method has permitted an increase in the storage limit of RBC from 21 to 35 days. Therefore, it can be concluded that adenine supplementation and maintenance of glucose metabolism during storage at 4C have a beneficial effect on RBC viability.

Notwithstanding the beneficial effects of adenine, other studies have failed to show the dependency of RBC viability on ATP concentration. For instance, rejuvenated, stored RBC that have either normal or supernormal concentration of ATP, survive less well than fresh RBC(8,9). Thus, it appears that in addition to ATP other factors play a role in decreasing RBC viability during storage at 4C.

The studies reported here were designed to explore the role of one immunological variable in the loss of RBC viability during storage. We hypothesized that C3 which was shown to attach to RBC progressively during liquid storage, might be important in the development of the preservation injury(10). C3 was implicated because a significant correlation was observed between the viability of stored human RBC and their content of RBC-bound complement(11), and also on the work of Brown et al who showed that rabbit RBC, sensitized with complement only, were rapidly sequestered in the liver, whereafter most returned to the circulation(12). However, a fraction of RBC were phagocytosed(12). Similarly, it has been shown that the

preservation injury applies only to a fraction of RBC(13). Our goal was to determine whether prevention of the attachment of C3 to RBC during storage at 4C would improve the viability of RBC. Since these studies required unorthodox manipulation of RBC prior to measurement of RBC survival in-vivo, an animal model was needed. The rabbit model was selected since these animals are readily available and relatively inexpensive.

In order to perform the investigation, we had first to validate the model by comparing the changes occurring in rabbit RBC during liquid storage to those in human RBC. The second objective of the study was to determine whether by preventing the attachment of C3 to the RBC membrane during 4C storage improved RBC viability.

Approach to the Problem

In order to determine whether the attachment of C3 to RBC membrane during blood storage at 4C contributes to the preservation injury, it was necesary to store RBC at 4C under such conditions that C3 would not accumulate on cell membrane. We planned to compare the viability of these RBC to similarly stored RBC that were sensitized with C3. Since the preservation injury can be evaluated only in-vivo, it was necessary to measure the 24-hour survival of stored RBC.

Since it was expected that in order to prevent the attachment of C3 to RBC, it would be necessary to manipulate the donated blood in ways not approved by the FDA, the study of human RBC was not acceptable. An animal model for in-vivo studies was required. Some RBC preservation work is done in primates(13). However, the use of the primate model is expensive and requires specialized facilities to take care of these animals. We decided to investigate whether a rabbit model would be suitable since the use of this animal is relatively inexpensive and these animals are readily available. The rabbit model seemed reasonable also on the basis of our preliminary experiments that showed C3 deposition to RBC membrane during storage at 4C of rabbit citrate-phosphate-dextrose (CPD) anticoagulated blood.

Our initial goal was to validate the rabbit model for studies of RBC preservation in-vivo. It was felt that this could be done if during liquid storage rabbit RBC undergo similar storage injury as do human RBC, i.e., that the 24-hour recovery in-vivo of stored RBC declines as a function of their length of storage at 4C. It has been shown in the human system that RBC rendered nonviable during storage are removed rapidly from the circulation(1,14,15). Although a large proportion of nonviable RBC may be eliminated within minutes of the injection, it has been shown that all nonviable RBC have been eliminated within the period of 24 hours(1,15,16). Support for this concept comes from the long-term survival data of stored RBC: RBC that are recovered in-vivo at 24 hours after transfusion have normal lifespan in normal recipient in the absence of any immunological incompatibility (16). For those reasons, 24 hour in-vivo survival has been used as a convenient measure of the viability of stored RBC. We wanted to show in the rabbit system that 24 hour survival in-vivo is also a valid measure of RBC viability.

In the human system, the percent survival of preserved RBC has been determined in one of two ways: 1) the recovery of 51 Cr labeled RBC approximately three minutes after transfusion is considered to be the 100% value and the recovery thereafter is taken as a percent of this value(17); 2) patient's RBC mass is determined independently by a radioactive trace label so that the

actual percent recovery at 0-time can be calculated exactly(14,15). It seems to be the consensus of the research community at present that the second method is the only correct one since damaged RBC can be removed during the first circulation(18). Thus, we elected to use the method #2 in rabbits and measure their RBC mass just prior to transfusion.

Our work under this contract was divided into the following categories:

Development of a rabbit model to evaluate RBC preservation at 4C

1. Technical Aspects
   a) collection and storage of blood
   b) measurement of RBC survival
   c) development of a method that avoids C3 deposition onto RBC membrane during storage at 4C

2. Effect of blood storage at 4C in citrate-phosphate-dextrose (CPD) on:
   a) C3 accumulation
   b) ATP, 2,3-DPG
   c) 24 hour survival

3. Effect of blood storage at 4C in inactivated CPD plasma on:
   a) C3 accumulation
   b) ATP, 2,3-DPG
   c) 24 hour survival

4. Comparison of 24 hour survival of stored RBC with and without C3

Methods

1a) Collection and Storage of Blood

In the initial experiments, about 10 ml of whole blood was collected from the central ear artery of a rabbit into 1.4 ml CPD anticoagulant (Fenwal Laboratories, Deerfield, IL). These samples were stored for various periods of time at 4C prior to performing RBC survival studies in the autologous animal. It soon became evident, however, that a large fraction of these samples were bacterially contaminated and could not be used for RBC survival studies. Furthermore, since there was only a small volume of blood available, it was difficult to perform all necessary in-vitro assays on these samples. An alternative approach using homologous transfusions was therefore devised.

Collection of donor blood: A donor rabbit was immobilized on a restraining board. Approximately 5" x 5" area on the anterior chest wall was shaved using a safety razor contained in a sterile prep kit. Thereafter, the area was washed with Betadine surgical scrub solution (Purdue-Frederich, Norwalk, CT) and the area was draped with sterile towels. Prior to performing the cardiac puncture, the site was once more cleansed with an alcohol swab. The heart cavity was entered with a 50 ml syringe equipped with an 18 gauge needle and containing 2 ml of diluted heparin (40 units/ml; L heparin, AlH2 Robins, Richmond, VA). About 48 ml of whole blood was drawn whereafter the syringe was removed and the blood was transferred into a 150 ml plastic bag (Fenwal Laboratories) containing 11 ml of CPD anticoagulant. Another 50 ml syringe was connected to the needle and a volume of 30 to 48 ml of whole blood was drawn and added to the plastic bag.

Further processing of the donor rabbit blood

Following collection the blood was centrifuged in a RC-3 centrifuge (Sorvall, Norwalk, CT) at 4C at 4200 rpm for seven minutes. The subsequent procedures were done under a clean air hood. The supernatant plasma was expressed to another plastic bag and divided into two equal parts. The packed RBC (volume about 30 ml) were batch-washed three times (each wash was done with about 167 ml of sterile 0.9% NaCl). After the last wash most of the supernatant fluid was expressed out of the bag and the RBC were divided into two equal aliquots. Half of the original CPD plasma was added to one aliquot of RBC, and the remaining treated plasma was added to the other aliquot of RBC. After recombining RBC and plasma, the average hematocrit was about 35%.

Thereafter, both units were stored at 4C for up to 21 days.

Blood cultures were done weekly by removing a 2 ml aliquot of blood(19). One ml of blood was added to each of two Bactec vials (Johnston Laboratories, Cockeysville, MD); one for aerobic and the other for anaerobic cultures. The vials were incubated at 35C for up to seven days. The aerobic cultures were placed on a shaker for the first two days. The cultures were read three times during the first two days, thereafter daily. If any of the cultures became positive, the study was discontinued.

1b) Double 51 Cr method of measuring survival of stored RBC(20)

Preparation of samples:

Fresh blood: Two ml of whole blood was collected from the central ear artery of the rabbit with a heparinized syringe and the contents were evacuated into a 3 ml heparinized test tube containing 100 USP units of Na heparin (B-D, Rutherford, NJ). About 10 uCi of 51 Cr (sodium chromate, New England Nuclear, Boston, MA) was added into the test tube and the tube was incubated at room temperature for 30 minutes mixing occasionally.

Thereafter, the RBC were washed three times with sterile 0.9% NaCl and resuspended into a total volume of about 2 ml in 0.9% NaCl. An accurate volume of one ml was drawn into a tuberculin syringe for injection. The remainder of the sample was prepared to be counted for radioactivity.

Stored blood: Two ml of blood was removed aseptically from the stored unit and placed into a sterile test tube together with about 80-100 uCi 51 Cr.

The blood and 51 Cr were incubated at 22C for 30 minutes with occasional mixing.

Then the labelled RBC were washed three times with sterile 0.9% NaCl and resuspended into a total volume of approximately two ml in 0.9% NaCl. An accurate volume of one ml was drawn into a tuberculin syringe for injection. The remainder of the sample was prepared to be counted for radioactivity.

Injection of the blood samples into the recipient rabbit and collection of the post-transfusion blood samples

The marginal ear vein of the rabbit was entered with a 21 gauge catheter (Minicath prn, Intermittent injection set, Desert Co., Sandy, Utah). The labeled fresh blood was injected completely. Thereafter, about 5 ml of sterile 0.9% NaCl was injected to clear the catheter of RBC. Exactly 30 minutes later a two ml heparinized blood sample was collected from the vein in the other ear. Thereafter, the one ml of stored blood was

injected through the catheter as described above.

The second blood sample was collected 30 minutes after this injection. Subsequent blood samples were collected 24 and 48 hours later and, if possible, three, five and seven days later.

Preparation of the blood samples for counting

A 1/25 dilution was prepared from the labelled, washed samples of the injected fresh and stored blood. A one ml aliquot was pipetted into a counting tube in duplicate.

One ml aliquots were also pipetted from the post-infusion samples. Spun hematocrits were done in triplicate in these samples.

Calculations

A = the total net CPM injected in the fresh samples = 25 x (CPM(fresh std) - BG)

B = the total net CPM injected in the stored sample = 25 x (CPM(stored std) - BG)

BG = background count

For each post-injection sample, we calculated net counts/ml packed RBC as follows:

$$\text{net CPM/cc PC} = \frac{\text{CPM (whole blood)} - \text{BG}}{\text{Hct}/100}$$

For each sample, this value was coded with the following notation:

CPM(0-fr)for the sample obtained 30 minutes after fresh blood injection

CPM(0-st) for the sample obtained 30 minutes after stored blood injection

CPM(24 hours) in the 24 hour sample post-injection

CPM(48 hours) in the 48 hour sample post-injection; etc.

$$\text{RBC mass} = \frac{A}{\text{CPM(0-f)}}$$

Survival at 0-time =

$$100\ \frac{\text{(RBC mass)x(CPM(0-st)-CPM(0-f))}}{B}$$

% survival at 24 hours =

$$\frac{100\ \text{(RBC mass)xCPM(24hours)-0.92xCPM(0-f)}}{B}$$

% survival at 48 hours =

$$\frac{100\ \text{(RBC mass)x(CPM(48 hours)-0.84xCPM(0-f)}}{B}$$

$$\% \text{ survival at 3 days} = \frac{100\ (\text{RBC mass}) \times (\text{CPM}(3\ \text{days}) - 0.80 \times \text{CPM}(0\text{-}f)}{B}$$

$$\% \text{ survival at 5 days} = \frac{100\ (\text{RBC mass}) \times (\text{CPM}(5\ \text{days}) - 0.66 \times \text{CPM}(0\text{-}f)}{B}$$

$$\% \text{ survival at 8 days} = \frac{100(\text{RBC mass}) \times (\text{CPM}(8\ \text{days}) - 0.62 \times \text{CPM}(0\text{-}f)}{B}$$

1c) Treatment of plasma to avoid C3 uptake by RBC during 4C storage

The separated rabbit CPD plasma was incubated at 56C or 60C for 60 and 120 minutes and recombined with washed rabbit RBC and stored for up to 14 days. The uptake of C3 by RBC was measured by an autoanalyzer hemagglutination method (10) using 1% PVP (polyvinylpyrrolidone, Technicon Corp., Ardsville, NY) and 1/2500 dilution of goat anti-rabbit C3 (lot #14401E; Cappel Laboratories, Westchester, PA).

2a) Effect of blood storage at 4C on C3 uptake by rabbit RBC

Four donor rabbit units were stored in CPD plasma at 4C for up to 29-31 days. Samples were collected daily, if possible, and tested for the uptake of C3 with the above mentioned autoanalyzer method.

2b) Effect of blood storage at 4C on ATP and 2,3-DPG content of rabbit RBC

Three donor rabbit units were stored at 4C with CPD plasma for up to 24 to 28 days. Blood samples were collected daily, if possible, and perchloric acid filtrates were prepared by a previously described method(21). ATP and 2,3-DPG were assayed in these samples by Dr. N. Fortier using a previously described method(21).

2c) Effect of blood storage at 4C on 24 hour survival of rabbit RBC. Determination of t 1/2 51 Cr of fresh rabbit RBC

In eight rabbits, using autologous, freshly collected RBC, 51 Cr survival studies were done as described above.

Blood samples were collected one day later from eight rabbits, two days later from seven rabbits, five days and eight days later from four rabbits. The mean percent recovery at various time periods was plotted on semilog paper and the best fit for the regression line was found by least squares analysis.

3a-3c: The effect of blood storage at 4C in heated CPD plasma on C3 accumulation, ATP, 2,3-DPG content and on 24 hour survival was performed as described in sections 2a - 2c except that the RBC had been stored in inactivated CPD plasma.

4. The comparison of the 24 hour survival of rabbit RBC with bound C3 to those without bound C3

Ten 51 Cr survival studies were done using homologous rabbit RBC that had been washed after collection, recombined with CPD plasma and then stored, on the average, at a hematocrit of 35% at 4C for seven days.

Ten 51 Cr survival studies were done using homologous rabbit RBC that had been preserved as above with the exception that the RBC had been recombined with heated CPD plasma after washing. The average percent survival at 24 hours after transfusion in the two groups was computed and the significance of the difference between the means was evaluated by the Student's t-test (R).

Six 51 Cr survival studies were done using homologous rabbit RBC that had been washed after collection, recombined with CPD plasma and then stored, on the average, at a hematocrit of 35% at 4C for 14 days.

Six 51 Cr survival studies were done using homologous rabbit RBC that had been preserved as stated above with the exception that the RBC had been recombined with heated CPD plasma after washing. The statistical analysis comparing the RBC survival in the last two groups was as described above.

Six 51 Cr survival studies were done using homologous rabbit RBC that had been washed after collection, recombined with CPD plasma and then stored, on the average at a hematocrit of 35%, at 4C for 21 days.

Five 51 Cr survival studies were done using homologous rabbit RBC that had been preserved as stated above with the exception that the RBC had been recombined with heated CPD plasma after washing. The statistical analysis comparing the RBC survival in the last two groups was as described above.

Since the RBC from one donor rabbit had been stored in both CPD plasma and inactivated CPD plasma, the ATP values of the transfused RBC were compared by the paired t-test to see if ATP

concentration of RBC differed in the paired units(22).

Results

51 Cr survival studies using fresh autologous RBC

The survival curve of fresh autologous rabbit RBC is shown in Figure 1. The y-intercept is 93.8% and the average measured 24 hour survival is 91.3%; the t 1/2 is 12.4 days when the % survival values up to eight days after transfusion were included in the analysis.

51 Cr survival studies using stored homologous RBC

Four long-term 51 Cr survival studies on homologous rabbit RBC stored for 21 days are shown in Figure 2. The recovery of RBC at 0-time varied from 90 to 100% and the recovery at 24 hours varied from 63 to 87%. The t 1/2 values ranged from 11 to 13.86 days (average 12.6 days).

Not all long-term homologous survival studies had normal t 1/2 values. In some cases, all the RBC had disappeared from the circulation by the eighth day, and others had shortened t 1/2 values, indicating that these animals had developed antibodies to the transfused RBC.

STUDIES ON RBC STORED IN CPD PLASMA

Effect on RBC-bound C3

Figure 3 shows that the percent agglutination of rabbit RBC with anti-C3 antibody increased as a function of blood storage.

Effect on the ATP concentration

Figure 4 shows the rate of decline of ATP concentration in rabbit RBC stored for up to 28 days. On the average, ATP had declined to half of its original value in 18 days. The ATP values of the unstored RBC in the units studied were 5.11, 5.40 and 5.97 umol/gHb.

Effect on RBC 2,3-DPG

Figure 5 shows the rate of decline of 2,3-DPG concentration in rabbit RBC stored for up to 24 days at 4C in CPD plasma. On the average, the concentration of 2,3-DPG had declined to half of the original value in about 18 days.

Effect on 24 hour survival

The average 24 hour survival of fresh blood was 91.35 +- 4.48% (mean +- 1 S.D., n=8). The average 24 hour survival of RBC stored for seven days at 4C was 86.1 +- 11.0% (mean +- 1 S.D., n=10). The average 24 hour survival of RBC stored for 14 days at 4C was 90.02 +- 6.40% (mean +- 1 S.D., n=5), and the average 24 hour survival of RBC stored for 21 days was 78.4 +- 17.8% (mean +- 1 S.D., n=5).

Method to prevent the uptake of the third component of complement, C3, by RBC during storage of blood at 4C

Freshly collected RBC were washed and then recombined with plasma that had been heated at 56C for one or two hours. Following storage of blood at 4C for 14 days, the RBC were coated with C3 to the same degree as RBC stored in CPD plasma.

Freshly collected RBC were washed and then recombined with plasma that had been heated at 60C for either one or two hours. The uptake of C3 by RBC was inhibited after storage of RBC at 4C for 14 days in the plasma heated at 60C for two hours but not if they had been stored in plasma heated at 60C for one hour.

Subsequently, to prevent C3 uptake, RBC were stored in plasma that had been heated at 60C for two hours. The percent agglutination of the stored RBC in the units studied varied from less than 1% to 12%.

STUDIES ON RBC STORED IN HEATED CPD PLASMA

Effect on ATP concentration

Figure 6 shows the rate of decline of ATP during blood storage at 4C. On the average, ATP declined to 50% of the original value by the 17th day. The intercept of the regression line was 75% of the original ATP concentration, indicating that in these units there was rapid drop of ATP concentration in the first few days. The initial ATP value in the two units studied were 3.74 and 5.62 umol/gHb.

Effect on 2,3 DPG concentration

Figure 7 shows the rate of decline of 2,3 DPG concentration during blood storage at 4C. In this unit studied, 2,3 DPG concentration had declined to half of its original value in 14.8 days.

Effect on 24 hour survival

The mean survival of RBC stored at 4C for seven days was 86.2 +- 15.2% (mean +- 1 S.D., n=10). The mean survival of RBC stored at 4C for 14 days was 81.5 +- 6.8% (mean +- 1 S.D., n=6), and the mean survival of RBC stored at 4C for 21 days was 72.2 +- 14.7% (mean +- 1 S.D., n=5).

Comparison of the effects of storage at 4C in CPD plasma and in heated CPD plasma

There was no significant difference in the ATP concentration in the paired units of blood that had been stored either in CPD plasma or heated CPD plasma (paired t-test = 0.263; p=NS).

Figure 8 shows the comparison of 24 hour survival of rabbit RBC stored for various periods at 4C either in CPD plasma or in heated CPD plasma. There was no significant difference in the RBC survival between these two groups of RBC at any storage period.

Discussion

An important goal of this research was to evaluate whether rabbit model could be used to study the preservation injury of RBC. Since these animals are readily available and relatively inexpensive, such a model would facilitate initial evaluation of new methods of preservation, and provide a tool to study the effects of different storage containers and methods of transportation. Furthermore, questions pertaining to the theory of RBC preservation could be tested in such a system.

We feel that the data presented supports the concept that the rabbit model utilizing homologous transfusions is suitable to study RBC preservation. As is the case with human blood donors, it was necessary to take great care in maintaining highly aseptic technics during collection of blood from donor rabbits, otherwise the blood was frequently shown to be bacterially contaminated. Furthermore, the collection of a rather large quantity of blood from a donor rabbit makes possible studies where multiple parameters can be evaluated simultaneously.

The survival studies were carried out using double 51 Cr technic(20). As has been shown in the human system, it is necessary to measure the RBC mass of the subject independently in order to evaluate the survival of stored RBC accurately since a fraction of the nonviable RBC can disappear from circulation prior to collection of the first blood sample(14,15). The data collected by us in rabbits tend to strengthen this concept (Figure 2). It has been shown in the human system that the RBC survival at 24 hours is a close approximation of the viability of the transfused RBC(1,15). This was also shown to be the case also in the rabbit model.

Figure 1 shows that the half time of the 51 Cr survival curve of fresh rabbit RBC is 12.4 days. The y-intercept of this survival curve is about 94% and the mean 24 hour survival was about 91%. Figure 2 shows that when stored RBC were studied, between 90% and 100% of them were recovered 30 minutes after transfusion. A fraction of the stored RBC had a rapid rate of destruction, but the rate of destruction after 24 hours was the same as that of fresh RBC (since the t 1/2 were similar to those of fresh RBC). Some of the survival studies done with stored RBC showed more rapid rate of long-term destruction or even so called "collapse" curves(1). These most likely were the result of immunization to the homologous blood. The frequency of such immunization could be minimized if the animals used as donors and recipients would be closely relateld. However, it is most unlikely that the 24 hour survival would be affected by immunization of the recipient rabbit to RBC of donor rabbit since

the period between the antigenic stimulation and development of antibodies is longer than 24 hours(23).

Our studies showed that the decline of ATP concentration in rabbit RBC during storage in CPD plasma at 4C was more rapid than that of human RBC. ATP concentration of rabbit RBC stored for 21 days was about 40% of the original value, whereas ATP of human RBC stored for equal amount of time is about 86% of the original value(24-26). On the other hand, the 2,3 DPG concentration in rabbit RBC was maintained somewhat longer than in human RBC. 2,3-DPG in rabbit RBC declined similarly in rabbit and human RBC(24-26) since ATP concentration in unstored rabbit RBC is higher than in human RBC, the absolute ATP concentration in rabbit and human RBC after 21 days storage is similar.

We showed that during liquid storage rabbit RBC bind the third component of complement to their membranes as do their human counterparts. Furthermore, the 24 hour survival of rabbit RBC stored in CPD plasma at 4C for up to 21 days (Figure 8) was very similar to the 24 hour survival of human RBC stored under similar conditions. These data indicate that liquid storage in CPD plasma induces both in rabbit and human RBC a storage lesion of similar magnitude. Thus, the study of the storage lesion in the rabbit RBC is expected to provide information that is applicable to the storage lesion in human RBC.

Using the rabbit model, our goal was to test the hypothesis that RBC-bound C3 causes the storage injury. To avoid C3 uptake by RBC during liquid storage, rabbit RBC were stored in autologous plasma that had been previously heated at 60C for two hours. RBC stored in such plasma did not lose ATP or 2,3 DPG at a more rapid rate than did the control RBC stored in CPD plasma. However, in the two units of blood in which we studied the rate of ATP depletion, ATP concentration appeared to decline more rapidly in the first few days than it did subsequently. This could have happened, for instance, if the heated plasma had not cooled sufficiently prior to combining it with the RBC.

The 24 hour survival of RBC stored in heated plasma was no better than that of control RBC stored in CPD plasma. This could indicate that either C3 was not critical to the development of the preservation injury, or that heated plasma provided such a harmful environement to the RBC during storage that the benefit of C3 removal was negated. It is possible that during heating at 60C, aggregates of immunoglobulins formed in plasma. These aggregates could have subsequently attached to RBC decreasing their survival. It is possible that attachment of C3 to RBC during storage is secondary to some other phenomenon that is actually the preservation injury. It has been shown that aggregates of immunoglobulins activate the alternative pathway of

complement(27). However, we don't know whether aggregation of immunoglobulins occurs during 4C storage. In order to delineate more clearly the role of RBC-bound C3 in preservation injury, RBC should not be suspended in heated plasma during storage to avoid C3 uptake. We are planning to store RBC in Adsol solution (Fenwal Laboratories) to maintain their ATP concentration and avoid C3 uptake. Control RBC will be stored in Adsol and plasma to permit C3 uptake. This approach will show more clearly whether RBC-bound C3 plays a role in the development of the preservation injury.

CONCLUSIONS

It was shown that rabbit RBC undergo similar changes during liquid storage in CPD plasma as human RBC. The concentration of ATP in fresh rabbit RBC was higher than that in human RBC while the rate of decline at 4C was more rapid in rabbit RBC. The concentration of 2,3 DPG was about two times higher in fresh rabbit RBC than in fresh human RBC and the rate of decline of this metabolite at 4C was similar in rabbit and human RBC. Rabbit RBC stored at 4C in CPD plasma were shown to take up C3 (the third component of complement) during storage. The viability of rabbit RBC after various periods of storage at 4C was very similar to that of human RBC, indicating that rabbit RBC developed the storage lesion at 4C in CPD plasma at about the same rate as human RBC. Furthermore, it was shown that the measurement of 24 hour survival is an acceptable way of quantitating the storage lesion in rabbit RBC since damaged RBC are removed rapidly within 24 hours and the remaining RBC have a normal long-term survival in the absence of immunological incompatibility. It appears, therefore, that the rabbit model is an easy, inexpensive way to evaluate the effectiveness of new preservation technics and the effect of other variables such as transportation, on RBC preservation. The rabbit model was also deemed suitable for basic research studies on RBC preservation.

In evaluating the role of RBC-bound C3 on the preservation injury, we stored RBC in heated plasma (60C for two hours) that prevented C3 uptake by RBC during storage. The viability of such RBC was no better than that of RBC to which C3 was attached. It was thought that the storage environemnt in heated plasma was damaging RBC. An alternative approach of storage in Adsol solution is suggested. This method of preservation permits maintenance of RBC ATP level but does not support C3 binding by RBC.

RECOMMENDATIONS

Rabbit model can be employed in studies involving RBC preservation to evaluate new technics and storage variables quickly and inexpensively.

Further studies to elucidate the nature of the preservation injury of RBC and the role of RBC-bound C3 in it are recommended. In these studies, we would employ storage in Adsol solution rather than in heated CPD plasma to avoid C3 uptake while maintaining RBC ATP concentration.

Figure 1

51 Cr survival of freshly collected rabbit RBC. The value at 24 hours is a mean of eight studies, that at 48 hours is a mean of seven studies, values at five and seven days are means of four studies.

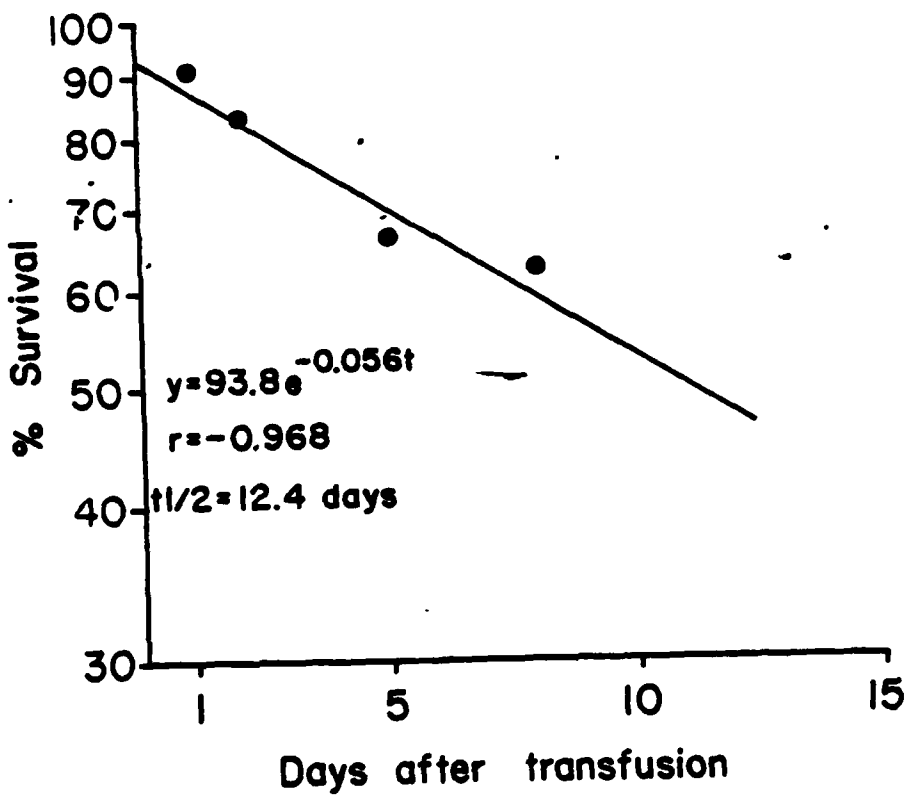


Figure 2

Four 51 Cr survival curves of homologous rabbit RBC, stored at 4C for 21 days.

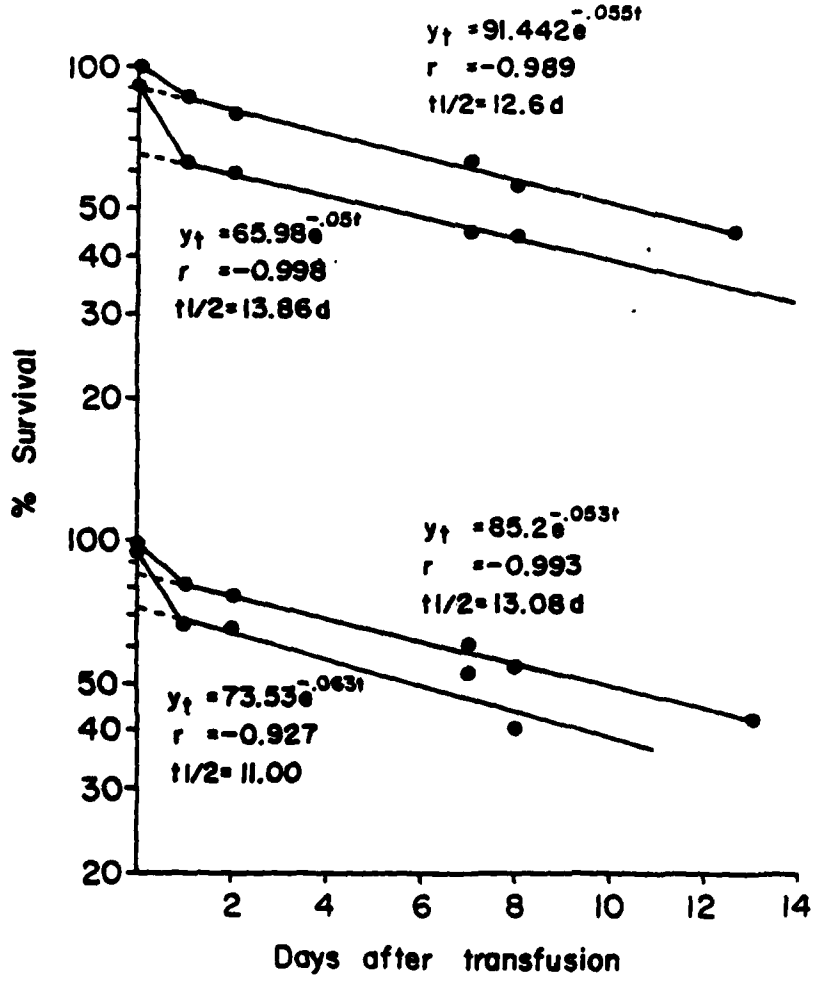


Figure 3

Percent agglutination of rabbit RBC with anti-C3, shown as a function of the length of storage. The increase in the % agglutination indicates that proportionally larger quantity of C3 binds to RBC the longer the blood is stored.

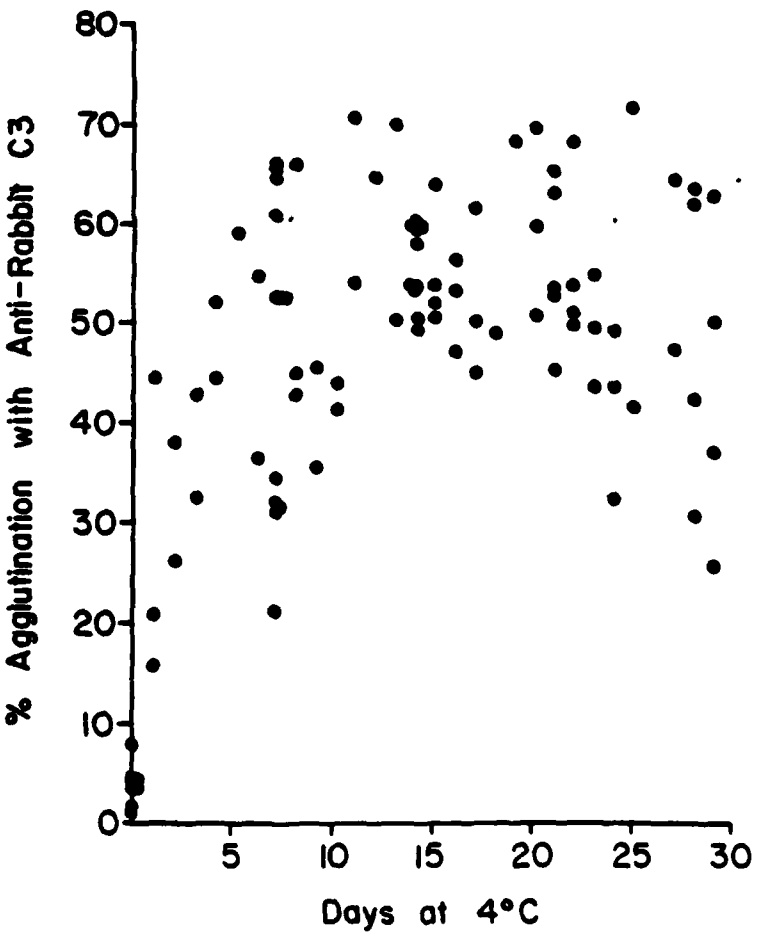


Figure 4

The rate of decline of ATP concentration in rabbit RBC that were washed with 0.9% NaCl immediately after collection, then recombined with CPD plasma and stored at 4C.

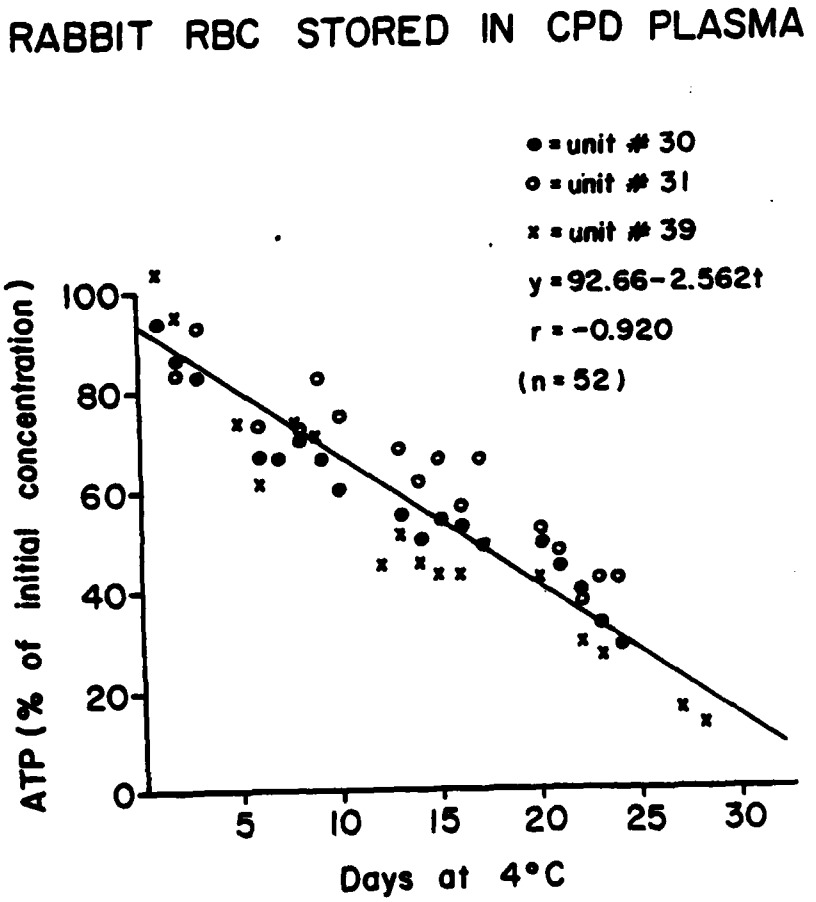


Figure 5

The 2,3 DPG concentration in rabbit RBC that were washed with 0.9% NaCl immediately after collection, then recombined with CPD plasma and stored at 4C.

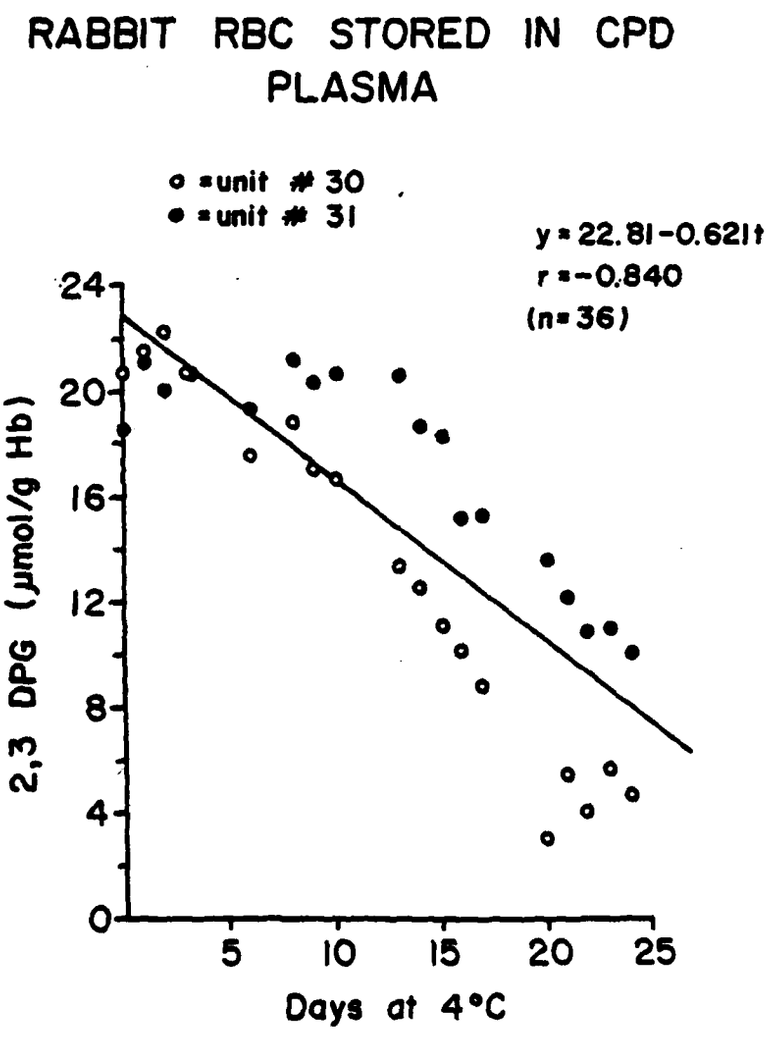


Figure 6

The rate of decline of ATP concentration in rabbit RBC that were washed with 0.9% NaCl immediately after collection, then combined with heated plasma (60C for two hours) and stored at 4C.

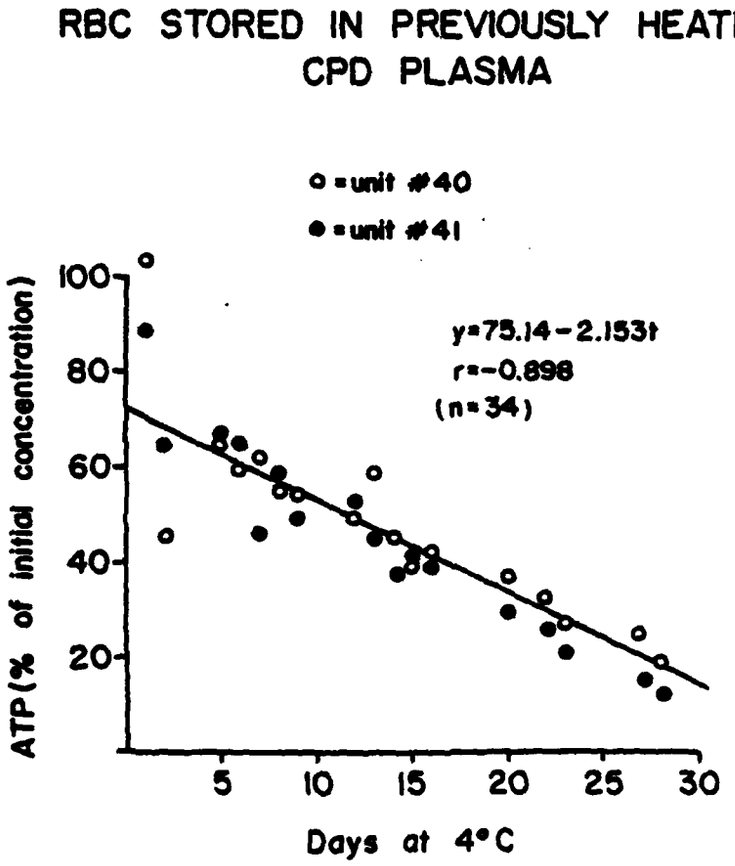


Figure 7

The 2,3 DPG concentration in rabbit RBC that were washed with 0.9% NaCl immediately after collection, then combined with heated plasma (60C for two hours) and stored at 4C.

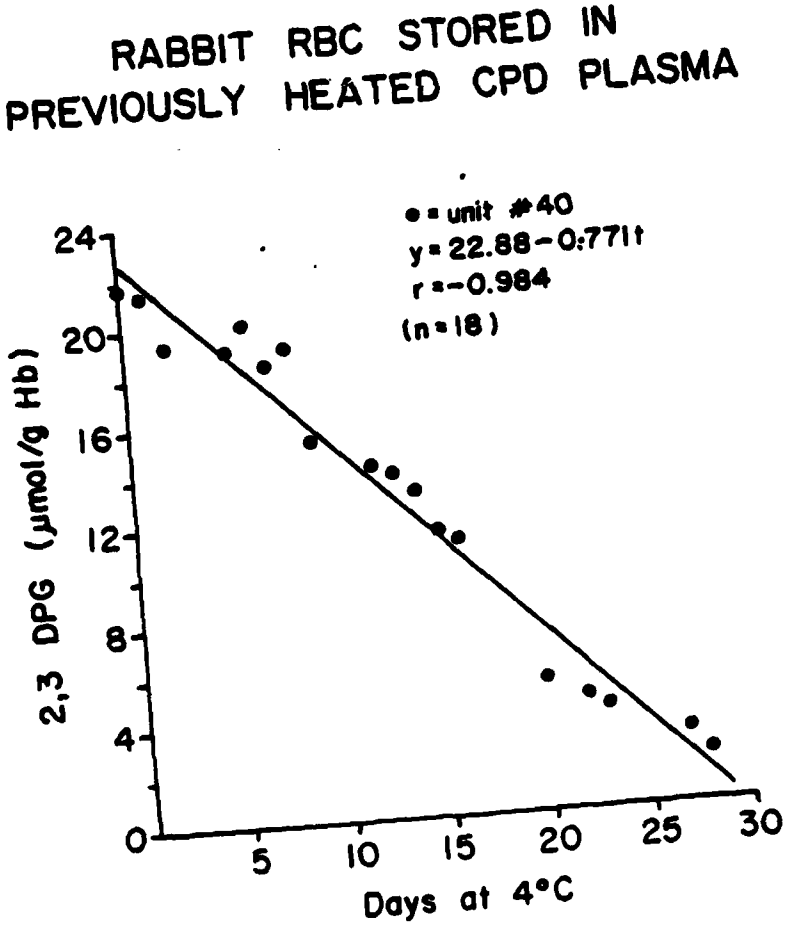
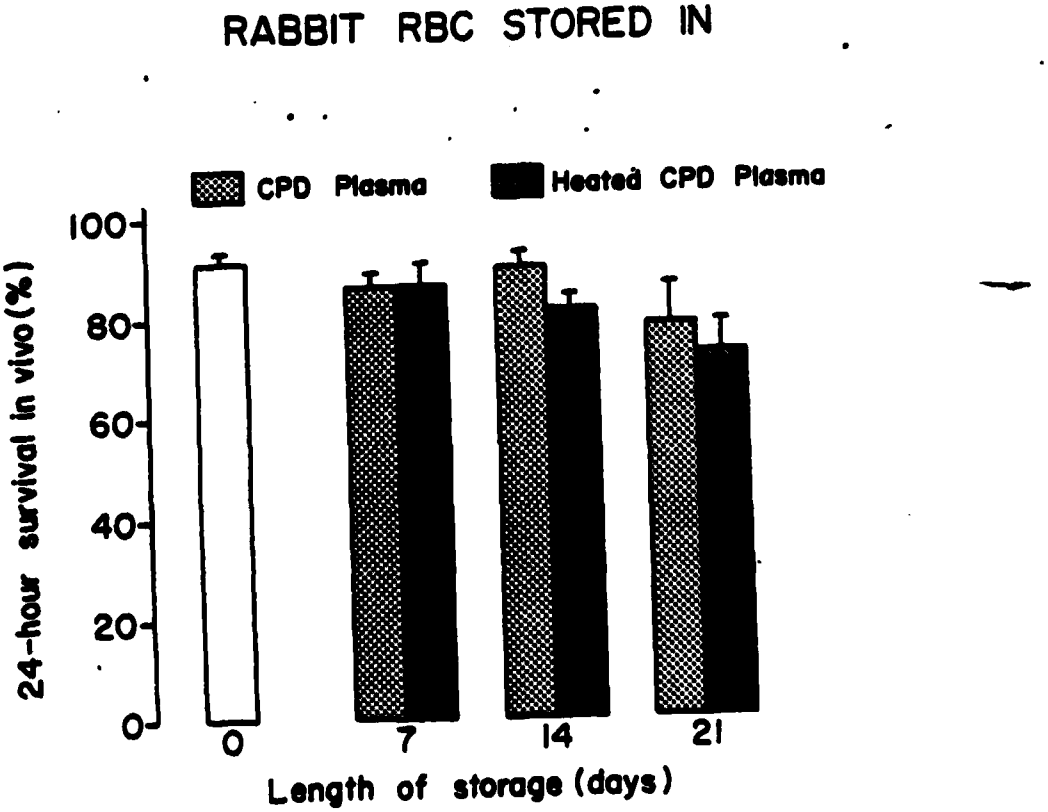


Figure 8

Comparison of the 24 hour survival of rabbit RBC that had been washed immediately after collection and then recombined with CPD plasma and stored for periods of 7, 14 and 21 days to the 24 hour survival of rabbit RBC that had been washed immediately after collection, combined with previously heated plasma (60C for two hours) and stored for periods of 7, 14 and 21 days. There was no significant difference in the 24 hour survival of the differently preserved RBC stored for seven days (t= -0.016, p=ns), for 14 days (t=1.92, p=ns) or for 21 days (t=0.537, p=ns).



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